

WEBVTT

NOTE duration:"00:56:57.3920000"

NOTE language:en-us

NOTE Confidence: 0.918851494789124

00:00:00.240 --> 00:00:24.370 Everyone we're going to get started if so, if anyone is out in the in the hallways or your friends can go grab them. So we could get the second half started so I am absolutely delighted to get to present Doctor Cornelia Trimble, who is a professor of gynecology obstetrics oncology and pathology so she actually did two separate residencies.

NOTE Confidence: 0.919537127017975

00:00:24.890 --> 00:00:44.530 Here and she's actually been illuminary in Women's Health, specifically in developing some of the preventative as well as the therapeutic vaccines for cervical cancer and actually last night over dinner. We were chatting how she's successfully putting herself out of business, so if the Doctor Trimble can come up thank you.

NOTE Confidence: 0.950687050819397

00:00:52.070 --> 00:00:56.490 Good afternoon and thank you in advance for your attention.

NOTE Confidence: 0.94004237651825

00:00:58.600 --> 00:01:00.940 And, of course for staying awake right after lunch.

NOTE Confidence: 0.833092331886292

00:01:02.850 --> 00:01:19.430 OK, as doctor legally know alluded to where I'm coming from is that I'm both a gynecological ethologist as well as an OBGYN and along the way I seem to have backed up into immunotherapy.

NOTE Confidence: 0.891192317008972

00:01:23.540 --> 00:01:52.200 And I focus on pre malignant disease because early on, it occurred to me that this patient population was going to be an informative one. It's a great time to be in tumor immunology. We're actually making progress. What we're doing is not anecdotal anymore. And here's Jim Allison is making us all feel as if or a part of it is really he's really been quite gracious about it with huge fun.

NOTE Confidence: 0.710525691509247

00:01:53.320 --> 00:01:54.610 Alrighty.

NOTE Confidence: 0.916472554206848

00:01:55.140 --> 00:02:22.780 20% of human cancers are caused by specific infections with a specific known pathogen so that raises the possibility of being able to either prevent disease or treat it by focusing persons immune response on an

pathogen in the case of HPV disease. We have known tumor specific antigenic targets the driver.

NOTE Confidence: 0.865809977054596

00:02:23.750 --> 00:02:36.270 Jeans if you will are viral nonself. These both of these uncle proteins are required functionally and functionally obligate manner for the initiation an persistence of disease.

NOTE Confidence: 0.908368408679962

00:02:37.810 --> 00:02:38.700 And we know.

NOTE Confidence: 0.866859674453735

00:02:40.940 --> 00:02:47.760 Anecdotaly that each PV specific T cell responses, indeed Ken eliminate some HPV malignancies.

NOTE Confidence: 0.896736025810242

00:02:48.480 --> 00:02:56.410 After that, we have the same todo list as everybody else OK. We have to figure out a tumor specific T cell response.

NOTE Confidence: 0.909241855144501

00:02:57.140 --> 00:02:59.750 OK then the T cells have to know where to go.

NOTE Confidence: 0.939046144485474

00:03:01.370 --> 00:03:04.070 Oh, and then they have to be able to function when they get there.

NOTE Confidence: 0.897828876972198

00:03:04.730 --> 00:03:18.170 And the kicker for HPV disease is all HPV malignancies occur or a rise in non sterile barrier epithelia. So you know that the rules of immune homeostasis are going to be really different in each site.

NOTE Confidence: 0.895182430744171

00:03:19.820 --> 00:03:29.650 I put this slide together so we can all start on the same page. All squamous cancers arise from an untreated high grade lesion.

NOTE Confidence: 0.895932137966156

00:03:30.330 --> 00:03:35.370 This is what a normal cervix looks like in 3 different ways.

NOTE Confidence: 0.901294052600861

00:03:36.120 --> 00:03:41.270 This is a cytology specimen. This is a low power H&T and this is what it looks like.

NOTE Confidence: 0.71162885427475

00:03:41.910 --> 00:03:42.660 Grossly.

NOTE Confidence: 0.920753419399261

00:03:43.480 --> 00:03:46.310 In the setting of a persistent HPV infection.

NOTE Confidence: 0.876028776168823

00:03:47.040 --> 00:04:06.170 You get soon 3 from 40,000 feet. You can see that in these cells is compared to those the nuclei are bigger right. Blue is bad, these nuclei take up most of his cell as opposed to a normal cells where the nucleus is just an anybody pinprick.

NOTE Confidence: 0.894872844219208

00:04:06.820 --> 00:04:16.390 This is what our Histology of a high powered high grade lesion looks like and you can see it looks really disorganized and ticked off compared to that.

NOTE Confidence: 0.894534468650818

00:04:16.890 --> 00:04:37.680 And when we diagnose high grade lesion. This is not rocket science. Actually, we put a dilute vinegar solution on the cervix and because these cells with hardly any cytoplasm or going to dehydrate much faster than these the lesion turns opaque when we look at it with a green filter very low tech.

NOTE Confidence: 0.858249843120575

00:04:38.220 --> 00:04:51.540 And In addition, I'll show you more of this later who looking for this really sharply demarcated Acedo White area. We look for a very pathognomonic pattern of neo vasculature cold mosaicism.

NOTE Confidence: 0.913305401802063

00:04:54.570 --> 00:04:55.540 In this setting.

NOTE Confidence: 0.312655210494995

00:04:56.150 --> 00:05:13.260 Of a persistence in 3, I should not be able to show you this last column in this day and age.

NOTE Confidence: 0.909108936786652

00:05:14.300 --> 00:05:24.260 And here's what it looks like when you just look at it and actually this is the only slide. The medical students ever remember 'cause they tell him it looks like a cheese pizza.

NOTE Confidence: 0.776730179786682

00:05:26.720 --> 00:05:27.540 No then.

NOTE Confidence: 0.909073770046234

00:05:28.710 --> 00:05:49.200 Having said that pre invasive cervical HPV disease is really incredibly indolent. We think that immune competent people that

transition from sin 3, too. Invasive cancer occurs over the time frame of 10 to 15 years. Of course, we don't know that, but retrospective studies.

NOTE Confidence: 0.880312323570251

00:05:50.000 --> 00:06:12.270 Suggest that and the relevant biological effect in sin 3 similar to other virally induced malignancies is that the HPV genome has integrated into the host genome and after that. These 2 uncle proteins. I told you about E 6 N B7. There expressed constitutive. Lee both in pre invasive as well as cancer.

NOTE Confidence: 0.908333420753479

00:06:14.640 --> 00:06:44.850 So when I started on the faculty. I had no idea what I was gonna do so. I went into Hunter Gatherer Mode since that's where I was on the food chain and and I observed I had an observation. Ull protocol in which I watched patients who had a fit biasing proven high grade lesion and I watched them for 15 weeks before doing their re section that is totally within the standard of care. It's in fact, the average amount of time it takes to work up a patient in Hopkins.

NOTE Confidence: 0.859442293643951

00:06:44.850 --> 00:06:56.270 And along the way 'cause I'm Taipei. We did an interval colposcopy. Even though the likelihood of progression was nil and it Week 15, we did a surgical resection.

NOTE Confidence: 0.934592485427856

00:06:58.580 --> 00:07:08.460 And then had a post up hundreds of women actually participated in this study and were very, very grateful to them because we learn some very useful things first of all.

NOTE Confidence: 0.912821412086487

00:07:08.980 --> 00:07:16.650 We compare the diagnostic biopsy to the Week 15 tissue resection 20% of these things were gone.

NOTE Confidence: 0.865731656551361

00:07:18.140 --> 00:07:19.270 At Week 15.

NOTE Confidence: 0.870663523674011

00:07:20.640 --> 00:07:22.130 How does that happen?

NOTE Confidence: 0.887655138969421

00:07:22.720 --> 00:07:38.840 Will along the way we have all of these different kinds of specimens as you can see here. So we can compare tissue micro environments or what have you in FFP sections from from weeks road with 15?

NOTE Confidence: 0.895592629909515

00:07:39.440 --> 00:07:51.450 We have peripheral blood so in these patients and in subsequent patients enrolled in Interventional trials with roughly the same schedule, we can look at.

NOTE Confidence: 0.914539813995361

00:07:52.140 --> 00:07:54.380 HPV specific immune responses.

NOTE Confidence: 0.803973317146301

00:07:55.190 --> 00:08:02.320 In the serum which we also have lanja tude only we can look for endosomes and cell free DNA.

NOTE Confidence: 0.856326341629028

00:08:02.970 --> 00:08:09.990 On the frozen tissue, which I also get when I do the reception, which they can do because I'm a surgeon.

NOTE Confidence: 0.904780268669128

00:08:10.720 --> 00:08:38.100 Is and have worked things out with our search path people we can do as you know a lot of things fresh frozen tissue compared to FFPE and because we also know already know what happens? What's happening in the frozen section if and only if the pathologist has no suspicion of invasion. We keep the bank Clock and we also take a sliver of fresh tissue from immediately adjacent.

NOTE Confidence: 0.877549111843109

00:08:39.440 --> 00:08:55.660 We got these brushes along the way I wasn't absolutely sure what we would do it, then we can do now. HPV genotyping but we all can also quantitate the microbiota and Aviram and the fungal because you know.

NOTE Confidence: 0.94874906539917

00:08:56.240 --> 00:08:58.070 This is not a sterile environment.

NOTE Confidence: 0.847622990608215

00:09:00.640 --> 00:09:05.340 What do we learn OK no lesions progressed during the study window?

NOTE Confidence: 0.921327948570251

00:09:07.110 --> 00:09:12.440 In some patients endogenous mechanisms could eliminate incipient cancers.

NOTE Confidence: 0.88071596622467

00:09:13.470 --> 00:09:15.140 OK light bulb moment.

NOTE Confidence: 0.922079503536224

00:09:16.260 --> 00:09:30.880 These incipient cancers were a great model if you will to learn how organs site specific characteristics enhanced malignant transformation from the pre invasive state.

NOTE Confidence: 0.832742810249329

00:09:31.960 --> 00:09:33.690 And permitted that to persist.

NOTE Confidence: 0.864595055580139

00:09:36.480 --> 00:09:55.320 At this point drew Mark Portal marched into my office. This is my only mouse lied and said you want to do HPV vaccines. I said, yeah seemed like a doable problem except like drew the only thing I remembered from immunology was that an antibody looked like a why.

NOTE Confidence: 0.804770767688751

00:09:57.050 --> 00:10:04.500 So Drew and TC, who had made the DNA vaccine, which was.

NOTE Confidence: 0.874536037445068

00:10:06.300 --> 00:10:26.400 Which housed a mutated HPV 16 sequence for E7? It was mutated in 2 sites so as to abrogate function? However, the configuration was similar enough to wild type, but T cells that were activated by this vaccine also recognized wild type and.

NOTE Confidence: 0.847039639949799

00:10:26.960 --> 00:10:37.820 These are mine's that got vaccinated, and these are mice who didn't this in the black 6 model. This vaccine was preventative as well as therapeutic.

NOTE Confidence: 0.944468080997467

00:10:39.250 --> 00:10:41.240 This is my first clinical trial.

NOTE Confidence: 0.924101889133453

00:10:41.960 --> 00:10:48.930 I basically did the mouse experiment and I gave successively higher doses of the DNA vaccine.

NOTE Confidence: 0.890347957611084

00:10:50.110 --> 00:10:58.330 This is one of those, 1 slide 3 years of work slides so anyway. The problem was nothing happened.

NOTE Confidence: 0.874193489551544

00:10:58.980 --> 00:11:21.610 Here, I have detected Elispot responses to E 7. These are in my observation. Ull patients at Week 0 Week 15, so you know exactly what's going on in that issue and these were isa responses to E 7 in non regressors. So you don't need a statistician to tell you they are the same.

NOTE Confidence: 0.881924092769623

00:11:23.350 --> 00:11:42.090 Here is where the immune responses that we saw in the vaccinated, patients and they overlap so the vaccine didn't make detectable immune responses and the rate of his so logic regression was exactly the same as it was in my observational cohort.

NOTE Confidence: 0.702124834060669

00:11:42.680 --> 00:11:43.110 Well.

NOTE Confidence: 0.899604022502899

00:11:44.570 --> 00:11:55.290 This is what we learn from that study and others peripheral blood immune responses to driver uncle proteins, which is couldn't find them in the peripheral blood.

NOTE Confidence: 0.914233326911926

00:11:58.430 --> 00:12:15.650 Well, maybe it was a function of where we were giving the vaccine. So we did. Another study testing Intradermal. Intralesional Yes, we invent projected the cervix and intramuscular administration of another therapeutic vaccine and again.

NOTE Confidence: 0.92198920249939

00:12:16.660 --> 00:12:22.970 Nothing happened so these are immune responses to E 7 in patients who got.

NOTE Confidence: 0.882345139980316

00:12:23.780 --> 00:12:34.900 Needle free powder mediated Intradermal vaccination. There's in the cervix and here is I am the red dots are regressors and you can see that.

NOTE Confidence: 0.881047308444977

00:12:35.440 --> 00:12:44.930 This is a real bummer and the Raiders regression again is the same as in my une Vaccinated Group. Let's do think these cells are dead. This is the positive control.

NOTE Confidence: 0.857928693294525

00:12:45.970 --> 00:12:53.590 OK, what was the lesson there, both need to find a vaccine strategy that immunogenic in people.

NOTE Confidence: 0.894189655780792

00:12:55.540 --> 00:13:00.110 And at that point I decided to put on my pathology hat.

NOTE Confidence: 0.939347267150879

00:13:03.950 --> 00:13:08.120 And you can see right here. You don't need to be a pathologist to see where the party is.

NOTE Confidence: 0.846828937530518

00:13:08.620 --> 00:13:09.930 This is sin 3.

NOTE Confidence: 0.906470835208893

00:13:10.490 --> 00:13:12.380 And this is adjacent normal.

NOTE Confidence: 0.924391508102417

00:13:12.940 --> 00:13:15.750 All those black polka dots are immune cells.

NOTE Confidence: 0.910978019237518

00:13:17.400 --> 00:13:37.690 So they all came to the right place. The problem is this dotted line is the basement membrane of epithelial part of the lesion and the problem is that all of us. She sells are on one side of that dotted line and the lesions on the other side, so these guys did search and forgot to do destroy I mean, they came there and they're all sitting there is singing Kumbaya.

NOTE Confidence: 0.892153203487396

00:13:39.510 --> 00:13:40.560 Why was that?

NOTE Confidence: 0.882392048835754

00:13:41.990 --> 00:14:04.910 Now then one method of immune invasion that we see pretty commonly with human solid tumors is the neo vasculature. Downregulates expression invitation molecules and so we wondered if that might be the case. Even in pre invasive disease. After all, we look for a very specific pattern of neo vasculature. Now then here's the lesion.

NOTE Confidence: 0.865760922431946

00:14:06.190 --> 00:14:27.300 The first order of business was to figure out what homing integrins were expressed on cervical T cells. I thought they were going to be expressing skin homing integrants 'cause. It's squamous epithelium, but neither none in the T cells in either normal cervix or in soon 3 as you can see Express' CLACC R4.

NOTE Confidence: 0.89534318447113

00:14:28.080 --> 00:14:46.200 In the 1st of many Bubba Gump moments the negative control look like this. Virtually all of the cervical T cells expressed A4 Beta, 7, both in normal normal tissue as well as in sin 3. So what's the next thing you do you go look for expression of the ligand.

NOTE Confidence: 0.834030449390411

00:14:46.710 --> 00:14:47.780 Which is mad cow?

NOTE Confidence: 0.881606161594391

00:14:50.460 --> 00:14:57.700 Here's a lesion that allowed CD 8 cells into it serial section. This is expression of Madcam.

NOTE Confidence: 0.892148077487946



00:14:59.960 --> 00:15:02.570 And in contrast, here's a lesion that.

NOTE Confidence: 0.893909931182861

00:15:03.750 --> 00:15:06.690 Kept them out and this is the expression of mad cow.

NOTE Confidence: 0.883427679538727

00:15:08.740 --> 00:15:17.270 We had to quantitate this you couldn't to see right in any case, we did. We developed pattern recognition software.

NOTE Confidence: 0.875223338603973

00:15:17.930 --> 00:15:43.020 Before there was such a thing and you can see that the expression of CD8I mean the intensity of CD8 correlated pretty directly with the expression of Madcam. We segregated into normal epithelium and stroma and in CIN epithelium and stroma. The tightest correlation was in CIN epithelium with the correlation was actually .88.

NOTE Confidence: 0.920249938964844

00:15:46.350 --> 00:16:00.910 Would that tell us if we made a therapeutic response, probably we should think about ways to activate the vascular endothelium as well. And it's good to be able to try more than one thing in a human trial. 'cause they take so long to do.

NOTE Confidence: 0.849034070968628

00:16:01.800 --> 00:16:11.740 And I did this work with 3 characters. You may know Rachel Clark at Harvard. Russell Yang, whose argynnis logic pathologists at Hopkins Anakim Young book was at Memorial.

NOTE Confidence: 0.767868280410767

00:16:13.770 --> 00:16:14.630 Now then.

NOTE Confidence: 0.875562906265259

00:16:15.210 --> 00:16:23.550 We actually found a vaccine that made an immune response in humans. It's housed in a Recon vaccinia vector.

NOTE Confidence: 0.881333470344543

00:16:24.180 --> 00:16:44.940 And for this next trial. We tested a heterologous prime boost using the DNA prime and then the vaccinia boost. Now then if the DNA was actually priming a response I would have we would have expected to see a bigger Delta in the response to E 7 compared to the response to E 6.

NOTE Confidence: 0.822494626045227

00:16:46.050 --> 00:16:47.670 And away we go.

NOTE Confidence: 0.878106355667114

00:16:48.540 --> 00:17:03.590 These are the first three treatment groups. They all got the same type. Oh no no sorry same dose of DNA and then they got successively higher groups coming doses of the Combinant Vaccinium.

NOTE Confidence: 0.616236627101898

00:17:07.520 --> 00:17:08.090 And.

NOTE Confidence: 0.853369176387787

00:17:10.020 --> 00:17:22.420 After the first half of patients. These are in Noah Macomb. No alteration vaccine alone patients. We did see immune responses that were measurable without extensive ex vivo tinkering.

NOTE Confidence: 0.880257368087769

00:17:23.300 --> 00:17:26.920 That's a really hard step actually.

NOTE Confidence: 0.884810507297516

00:17:27.440 --> 00:17:29.560 But we had a runner on base.

NOTE Confidence: 0.929520785808563

00:17:31.340 --> 00:17:42.270 Now then we wanted to know of course, whether tinkering with the micro environment directly could improve the efficacy of therapeutic vaccine vaccination.

NOTE Confidence: 0.856587529182434

00:17:43.110 --> 00:18:12.040 So simultaneously we were enrolling patients and cohort that got just Amico Modelon on the cervix in medical models. You may know is a topical. TLR 78 agonist and we use it all the time. Externally we use it to treat warts. We use it to treat small basal cell. Carcinoma's and small in case active Nick Keratosis. We have a lot of experience is I don't know if any of you have ever used it but it scares up a really ferocious.

NOTE Confidence: 0.908241927623749

00:18:13.220 --> 00:18:20.150 Innate response wherever you put it now, then all of these patients had HPV 16 associated disease.

NOTE Confidence: 0.933854937553406

00:18:20.680 --> 00:18:28.640 It turns out that HPV 16 associate is associated with about 60% of high grade lesion So what does that mean?

NOTE Confidence: 0.903800666332245

00:18:30.890 --> 00:18:38.790 That means 40% are associated with other Genotypes and I had all these patients crying in my office because they nickel MoD is.

NOTE Confidence: 0.91892671585083

00:18:39.630 --> 00:18:55.000 Virus agnostic if you will, I opened up another treatment group for people who had high grades that were caused by HPV. Geno types other than 16 and the short version is the rate of regression in this group is so high that I've had to double the size of that cohort.

NOTE Confidence: 0.886624693870544

00:18:56.130 --> 00:19:03.220 Now then the last group got both the highest dose of vaccination as well as imiquimod applied directly to the cervix.

NOTE Confidence: 0.920829117298126

00:19:04.650 --> 00:19:07.080 We saw something so striking.

NOTE Confidence: 0.927023649215698

00:19:08.620 --> 00:19:15.880 In these vaccinated, patients that we published these results halfway through the trial actually So what I'm going to show you is.

NOTE Confidence: 0.903606772422791

00:19:17.240 --> 00:19:27.980 Dinner from these patients who got only vaccination Noah McElman and I've put on the Top right corner expected rate of spontaneous regression just in case you forgot it.

NOTE Confidence: 0.929580628871918

00:19:30.900 --> 00:19:32.980 One big question in the field with.

NOTE Confidence: 0.930102467536926

00:19:34.040 --> 00:19:37.160 Can peripheral vaccination make an immune response?

NOTE Confidence: 0.879943430423737

00:19:37.680 --> 00:19:39.180 That goes we're supposed to.

NOTE Confidence: 0.90169757604599

00:19:40.060 --> 00:19:41.200 And we think so.

NOTE Confidence: 0.939157128334045

00:19:42.810 --> 00:19:48.470 I'm going to show you subject matched tissue specimens from before and after vaccination.

NOTE Confidence: 0.892180562019348

00:19:49.970 --> 00:19:52.960 Here's one patience lesion before vaccination.

NOTE Confidence: 0.795531213283539

00:19:53.550 --> 00:19:54.430 Here's after.

NOTE Confidence: 0.792506039142609

00:19:56.790 --> 00:19:58.270 Here's Tibet before.

NOTE Confidence: 0.829373240470886

00:20:00.600 --> 00:20:01.560 So their killers.

NOTE Confidence: 0.879016220569611

00:20:02.400 --> 00:20:04.460 This is a slide this next set just.

NOTE Confidence: 0.901890516281128

00:20:05.000 --> 00:20:21.490 Made my hair stand on end for some reason I did Ki 67, which as you know is a proliferation marker the high grade lesion itself is a nice internal control because it's proliferating now then here is the same patient before and here she is after.

NOTE Confidence: 0.869419157505035

00:20:23.130 --> 00:20:32.700 The only reason that a T cell or at least the Top five reason that a T cell starts for live rating is if it's been activated by its cognate antigen.

NOTE Confidence: 0.90818452835083

00:20:35.000 --> 00:20:51.030 Now the not only were there T cells. There they organized into tertiary lymphoid structures that localized specifically to residual displeasure. If there wasn't so those of you are still awake, maybe thinking? What the hell is. She talking about excuse me that's normal.

NOTE Confidence: 0.901666283607483

00:20:51.630 --> 00:21:00.690 Well, what this is actually that thing in the middle that looks like a germinal center. That's the tip of a gland the rest of the gland is not in the plane of section.

NOTE Confidence: 0.883026897907257

00:21:02.720 --> 00:21:04.130 Looks like a German center.

NOTE Confidence: 0.897492289543152

00:21:05.260 --> 00:21:13.030 Now then for those of you who are not pathologists. Can you appreciate that this white blob book is kind of different from that white?

NOTE Confidence: 0.576091408729553

00:21:14.460 --> 00:21:14.810 Right.

NOTE Confidence: 0.825276672840118

00:21:15.450 --> 00:21:17.730 This one's not as blue.

NOTE Confidence: 0.788836181163788

00:21:18.280 --> 00:21:21.400 You want to get really the reason it.

NOTE Confidence: 0.894836485385895

00:21:22.320 --> 00:21:28.510 Looks interesting is because that's the sin 3 at the tip of the gland and that's the germinal center.

NOTE Confidence: 0.886567234992981

00:21:29.960 --> 00:21:34.270 Now then what pathologists do is we sit in the dark and we look at slides and we put them in piles.

NOTE Confidence: 0.902031242847443

00:21:34.900 --> 00:21:38.740 And then we look at him again put him in different piles like sorting baseball cards.

NOTE Confidence: 0.920785248279572

00:21:39.960 --> 00:21:46.200 So there's no way I could look at 12 of these things and not put together the same story in my head.

NOTE Confidence: 0.898414731025696

00:21:47.740 --> 00:21:52.690 It turned out that the frequencies of Clonally expanded debaters.

NOTE Confidence: 0.868440747261047

00:21:55.610 --> 00:22:04.390 With the frequencies the clonal T cells in the tissue were clonally expanded compared to blood from the same time point.

NOTE Confidence: 0.921586573123932

00:22:05.060 --> 00:22:07.630 This is within subject we did this with adaptive.

NOTE Confidence: 0.89865630865097

00:22:09.150 --> 00:22:17.250 This was really brute force, these are the Top 25 most frequent TCR's RV betas in the tissue.

NOTE Confidence: 0.883520066738129

00:22:18.020 --> 00:22:26.010 In the same in the blood in a vaccinated, patient and this is what they look like in a non vaccinated patient.

NOTE Confidence: 0.862390637397766

00:22:26.530 --> 00:22:29.100 Overlapping a Chalais HPV 16.

NOTE Confidence: 0.898001074790955

00:22:30.750 --> 00:22:43.760 Well, I don't really know what to do with that, so I compared the betas in patients who have been vaccinated, and had overlapping HLA and here's tissue TC, Rs and two of those patients.

NOTE Confidence: 0.927302062511444

00:22:44.340 --> 00:22:48.950 I don't know what that means there's 55 and they drop out. It's so clean.

NOTE Confidence: 0.869255244731903

00:22:49.720 --> 00:23:03.790 Some in the blood as well, but in contrast, when you looked at when we looked at vaccinated. Patients have been vaccinated, patients and une vaccinated, patients who had overlapping HLA there's nothing there.

NOTE Confidence: 0.904188871383667

00:23:05.650 --> 00:23:16.950 None of this is conclusive it's all indirect. But overall, it does suggest it did suggest that T cells in the tissue were there by a process of selection and not by leakage.

NOTE Confidence: 0.820973932743073

00:23:18.370 --> 00:23:19.470 They had a

NOTE Confidence: 0.907249629497528

00:23:20.030 --> 00:23:27.170 Functional phenotype, so there, I go sitting in the dark again did laser capture microdissection of the same cases.

NOTE Confidence: 0.899019122123718

00:23:29.500 --> 00:23:35.240 And looked at the stroma subjacent immediately to residual CIN.

NOTE Confidence: 0.848197937011719

00:23:36.020 --> 00:23:47.820 And we did a immunology plate. This is in the paper in the supplementary section. But we had a whole panel in 96, well panel of immune related jeans.

NOTE Confidence: 0.911459684371948

00:23:49.750 --> 00:23:59.030 This is normal this is one vaccinated, sin 3 and this is the vaccinated, so you can see some differences that are starting to percolate out.

NOTE Confidence: 0.871920168399811

00:23:59.810 --> 00:24:14.010 Not only was it in the stroma, but we also actually saw it in the epithelial compartment and again. He had a TH one signature so these are all kind of walk like a duck quack like a duck so far.

NOTE Confidence: 0.873383104801178

00:24:15.790 --> 00:24:31.890 Not only were their TLS tertiary lymphoid structures in the vaccinated lesions. Let me show you some of the difference again. Here's here's a patient before yeah, actually showed me this before OK. So here's an age of any of her before vaccination.

NOTE Confidence: 0.883413374423981

00:24:32.880 --> 00:24:36.570 Here's a higher power mag of that area right there.

NOTE Confidence: 0.887812554836273

00:24:37.840 --> 00:24:41.100 Quiet this is what Greg was talking about it's cold.

NOTE Confidence: 0.936280727386475

00:24:41.610 --> 00:24:55.720 Now then after vaccination her cervix look like World War 3 and it's really, really hot. These guys are trying to organize and this again is the basement membrane. I want to draw your attention to something you can see.

NOTE Confidence: 0.897221386432648

00:24:56.240 --> 00:25:00.770 From low power this is her residual disease, I think it's.

NOTE Confidence: 0.921141088008881

00:25:01.390 --> 00:25:03.970 Maybe 30 cells across.

NOTE Confidence: 0.919711828231812

00:25:05.660 --> 00:25:11.690 But technically she didn't respond. Let me show you what that looks like in higher power.

NOTE Confidence: 0.933691442012787

00:25:12.640 --> 00:25:15.690 Again, this is the basement membrane?

NOTE Confidence: 0.89878523349762

00:25:18.270 --> 00:25:25.990 These are those vessels. I mean, they're really hopped up and annoyed and this is what high endothelial venules look like in a lymph node.

NOTE Confidence: 0.900349318981171

00:25:30.340 --> 00:25:32.160 The T cells are actually getting in.

NOTE Confidence: 0.916106224060059

00:25:35.590 --> 00:25:39.910 And I understand their students in the audience you see these shrinky cells.

NOTE Confidence: 0.900520145893097

00:25:41.970 --> 00:25:42.730 These guys.

NOTE Confidence: 0.873709380626678

00:25:43.270 --> 00:25:45.240 That's what apoptosis looks like.

NOTE Confidence: 0.903874039649963

00:25:47.030 --> 00:25:54.200 So what did we learn OK. These were all important things to know crucial vaccination can in fact change.

NOTE Confidence: 0.871159553527832

00:25:54.820 --> 00:25:58.780 The immune context in the target lesion, I know where to go.

NOTE Confidence: 0.869473934173584

00:26:00.610 --> 00:26:13.400 Using conventional endpoints, these patients would not have been successes. They did have detectable profile blood responses. It was not by X fold, it was no.

NOTE Confidence: 0.91639518737793

00:26:14.810 --> 00:26:21.610 And it also in these patients technically the relations didn't go completely away, So what we realized is that.

NOTE Confidence: 0.905848741531372

00:26:22.390 --> 00:26:35.470 Maybe it takes an immune response little bit longer to do its thing and we were actually censoring the tissue endpoint at Week 15. Afterwards, I'm ended the protocol designed for the students? What those shrinky things are doing.

NOTE Confidence: 0.874816536903381

00:26:36.000 --> 00:26:47.550 God bless the postdoc who did this, this is a scanning him of a killer T cell. This is actually a cervical cancer cell and what's going on in those shrinky things is this?

NOTE Confidence: 0.797390162944794

00:26:50.900 --> 00:26:53.180 Kiss of death.

NOTE Confidence: 0.838890433311462

00:27:04.300 --> 00:27:06.560 What do shrinky things look like it's kind of?

NOTE Confidence: 0.908360242843628

00:27:08.530 --> 00:27:10.100 I just wanted to make it real for you.

NOTE Confidence: 0.905893385410309

00:27:12.950 --> 00:27:43.100 So these insights actually informed the design of subsequent clinical trials in this population. I did a clinical trial with group called inovio testing. a DNA vaccine. That was given with electroporation. Turns out that the electroporation does make the DNA vaccine. Immunogenic we vaccinated. Send three patients at study with 04. We put the boost a little farther out and the tissue endpoint we waited until.

NOTE Confidence: 0.845818102359772

00:27:43.100 --> 00:27:44.940 Week 36.



NOTE Confidence: 0.681085646152496

00:27:46.050 --> 00:27:47.070 We cured.

NOTE Confidence: 0.899419128894806

00:27:47.910 --> 00:27:59.890 Nearly half the patients. This 49 point, something percent right here. This is vaccinated, patients and placebo. Now then we also wanted to know the.

NOTE Confidence: 0.905574202537537

00:28:01.180 --> 00:28:31.310 Frequency of concurrent histologic regression and loss of detectable virus that happened in most of the vaccinated, patients and you can see not in nearly so much of the placebo patients. Now then these 2 columns represent people who had mixed infections that included HPV 16 and this effect was even more striking in the HPV mono infection lesions. HPV 16 mono infections are the most difficult ones to clear.

NOTE Confidence: 0.907575905323029

00:28:32.560 --> 00:28:33.520 And here we have.

NOTE Confidence: 0.90267688035965

00:28:34.030 --> 00:28:58.050 A rate of histologic regression. It's over 50% actually compared to the placebo group. This is consistent with the what we saw in the observation. Ull cohort I told you about first and the rate of concurrent regression as well as viral clearance was spectacular. In these 16, one Oh infections compared to the placebo group.

NOTE Confidence: 0.887268960475922

00:28:58.880 --> 00:29:00.800 And the phase 3 is now on going.

NOTE Confidence: 0.902916014194489

00:29:02.690 --> 00:29:04.650 Well, that was really fast overview of.

NOTE Confidence: 0.921970427036285

00:29:05.320 --> 00:29:14.360 A lot of iterations of basically figuring out reasons for failure. And if the students are out there. That's how I've spent, most of my career so.

NOTE Confidence: 0.944136440753937

00:29:14.920 --> 00:29:16.710 Don't be discouraged.

NOTE Confidence: 0.896124005317688

00:29:17.930 --> 00:29:25.920 This is something completely new and different I mean, I was a T cell chauvinist because I was raised by that guy, but then.

NOTE Confidence: 0.858648061752319

00:29:26.470 --> 00:29:27.840 We happened upon this.

NOTE Confidence: 0.912088572978973

00:29:29.080 --> 00:30:00.040 Really crazy thing art estimate is a Chinese herbal Medison that has been used since 400 AD by my people to treat acute malaria and the person who figured out how it worked won the Nobel Prize for medicine in 2015. There are millions of people who are alive because of her we have given people art estimate or derivatives. Orally, as the intramuscular injection. We run it into them. Ivy and in kids who are younger than the age of 5.

NOTE Confidence: 0.906717419624329

00:30:00.040 --> 00:30:04.050 And can't keep food down, we give it to him up there little bumps in a Suppository.

NOTE Confidence: 0.936029434204102

00:30:04.920 --> 00:30:14.780 So over 2,000,000 people have been treated with some form of art estimate and so we know a great deal about the toxicity and pharmacokinetics.

NOTE Confidence: 0.544625580310822

00:30:16.520 --> 00:30:16.980 OK.

NOTE Confidence: 0.88030606508255

00:30:17.650 --> 00:30:23.960 So it turns out that art estimate is cyto toxic to many human solid tumor cell lines.

NOTE Confidence: 0.906922519207001

00:30:24.940 --> 00:30:26.900 We don't exactly totally know why.

NOTE Confidence: 0.902721583843231

00:30:29.690 --> 00:30:35.320 Of course, it was decided toxic to cervical cancer cell lines and it turned out, it was also cytotoxic.

NOTE Confidence: 0.747534155845642

00:30:35.930 --> 00:30:41.540 In a free clinical dog model this is Dick Schlegel's group.

NOTE Confidence: 0.779151916503906

00:30:42.070 --> 00:30:42.730 Here's a

NOTE Confidence: 0.874727964401245

00:30:44.220 --> 00:30:46.430 United papilloma virus.

NOTE Confidence: 0.829429507255554

00:30:47.030 --> 00:30:54.230 Lesion treated with the active form of art estimate. These are just like casing beliefs evolve our pictures, it's gone.

NOTE Confidence: 0.87747985124588

00:30:57.960 --> 00:31:01.650 Our first clinical trial of art estimate was.

NOTE Confidence: 0.899505853652954

00:31:02.420 --> 00:31:25.890 Liberating we formulated the Artesa Nate into vaginal Suppository zne with exactly the same formulation as we use for Terazol 7, which is what we use to treat yeast infections. It was the same formulation. Same applicator and these patients with biopsy confirmed high grade lesions came in at weeks 02 and 4.

NOTE Confidence: 0.905627489089966

00:31:27.390 --> 00:31:42.630 And the amazing thing to me about this trial. One of them is she my patient came in and had a negative pregnancy test we handed her five. A box of five of these things and she took it home and did it herself.

NOTE Confidence: 0.944652616977692

00:31:44.800 --> 00:31:49.000 The first treatment group was a low dose 50 milligrams.

NOTE Confidence: 0.907980144023895

00:31:50.450 --> 00:31:57.380 The next treatment group was the full dose. We planned to give one cycle each cycle is 5 consecutive nights.

NOTE Confidence: 0.871713042259216

00:31:58.130 --> 00:32:02.880 The second, the 3rd treatment group got the same dose but 2 cycles.

NOTE Confidence: 0.894112765789032

00:32:03.560 --> 00:32:07.130 And the 3rd treatment group got 3 cycles.

NOTE Confidence: 0.895890116691589

00:32:10.520 --> 00:32:27.380 Well halfway through the trial, it was so clear that this thing was working that we also opened an analogous trial for Ain just anal intra epithelial neoplasia. Now, if you ever need it a non surgical therapy Ain would be.

NOTE Confidence: 0.885969460010529

00:32:28.350 --> 00:32:42.660 The place to try it out so we're working up the manuscript. The short version is we cured. More than 2/3 of the CIN patients. The phase 2B will with any luck open after next Wednesday. That's R 30 days with the FDA.

NOTE Confidence: 0.895837485790253

00:32:44.620 --> 00:33:00.170 The Ain trial is already open and I can tell you anecdotally. It's early days but it's working and it seems to not require an intact immune system. We think it's mediating cell death directly.

NOTE Confidence: 0.893505752086639

00:33:03.050 --> 00:33:31.280 Of course we opened up a trial this one is now open we formulated the art estimate is that ointment and are using it to treat Vin Boulevard into apathy Liam Asia, even though these all share the same etiologic agent. The biology is very, very different. For example, in Vin if you respect and intra epithelial lesion. Even if you have clean surgical margins. There's a 50% chance that that thing comes back.

NOTE Confidence: 0.841058492660522

00:33:32.930 --> 00:33:37.650 Maybe all of us have this patient.

NOTE Confidence: 0.851714193820953

00:33:38.220 --> 00:33:56.450 We have, we see patients who are immune competent by any measure that we can measure and they had nasty. HPV 16 high grade so there diagnosed in the cervix. You do a cone, OK, the high grade comes back so you do another column.

NOTE Confidence: 0.888737261295319

00:33:57.410 --> 00:34:05.550 Comes back so you do a third cone at this point. There's no cervix left so when it comes back, these people get a radical Wertheim hysterectomy.

NOTE Confidence: 0.899203062057495

00:34:06.960 --> 00:34:20.360 And my first patient was who came to see me 'cause she was out of options. She had even had radiation through her cough and there, it was back at her at her apex. So we made a compassionate use protocol just for her.

NOTE Confidence: 0.913526594638824

00:34:21.310 --> 00:34:27.920 And she had been dealing with this repetitive high grade for 15 years well.

NOTE Confidence: 0.92194390296936

00:34:28.940 --> 00:34:34.270 It's been more than a year now, and she has still no detectable HV V.

NOTE Confidence: 0.897459924221039

00:34:35.290 --> 00:34:40.140 So we gave it to a second patient amended the protocol gave it to a second patient.

NOTE Confidence: 0.918464660644531

00:34:40.660 --> 00:34:57.960 Her disease has gone away. We never see. And so at this point. This is very unusual actually the FDA contacted me and said look. We don't want to see you every time you feel sorry for a patient. Can you just make a protocol that has a specific end and we'll just go from there?

NOTE Confidence: 0.918806433677673

00:35:00.670 --> 00:35:02.420 OK, that's what's new what's next.

NOTE Confidence: 0.947767078876495

00:35:06.150 --> 00:35:10.470 What does a successful immune response look like?

NOTE Confidence: 0.915941119194031

00:35:11.090 --> 00:35:15.140 We're very fortunate position to have a lesion.

NOTE Confidence: 0.894746661186218

00:35:16.130 --> 00:35:22.700 And no histologic context and know a lot of other things, from the same time points.

NOTE Confidence: 0.91115266084671

00:35:24.090 --> 00:35:33.300 So here's a patient that I've shown you before, and these are Elispot responses to vaccine antigens, 16 E 67 and 1867.

NOTE Confidence: 0.935657799243927

00:35:33.860 --> 00:35:36.970 Now before vaccination at times 0.

NOTE Confidence: 0.819451928138733

00:35:37.690 --> 00:35:42.460 This is what her biopsy look like that's cold like in England.

NOTE Confidence: 0.918851613998413

00:35:43.370 --> 00:35:51.220 You know, then at this time point, which is Week 15 when we did the Conization. This is after all, free vaccinations.

NOTE Confidence: 0.911242604255676

00:35:51.940 --> 00:35:52.890 She did this.

NOTE Confidence: 0.904788851737976

00:35:56.860 --> 00:35:59.560 So we can ask begin to ask questions now about.

NOTE Confidence: 0.921139359474182

00:36:00.290 --> 00:36:08.300 How these things happen? What are what's going on in a lesion micro environment that allows a transition from being cold?

NOTE Confidence: 0.589821457862854

00:36:09.150 --> 00:36:10.170 So being

NOTE Confidence: 0.836674213409424

00:36:10.970 --> 00:36:11.940 A party here.

NOTE Confidence: 0.890907526016235

00:36:14.450 --> 00:36:25.260 Are these T cells even HPV specific is so tempting to speculate that they might be? I mean there clonally expanded there getting into the lesion. We see Apoptosis, but those are all indirect.

NOTE Confidence: 0.910161316394806

00:36:30.920 --> 00:36:39.320 And I should say that we're collaborating with Kelly Smith, whom Drew mentioned she's using we're using her manifest assay to identify.

NOTE Confidence: 0.852138936519623

00:36:40.890 --> 00:36:53.490 HPV specific T cell receptors in the peripheral blood and once we've plucked those proteins. We stimulate them with overlapping proteins, then we look for those TC, R, tissue.

NOTE Confidence: 0.941383898258209

00:36:58.420 --> 00:37:00.280 In something that looks like that.

NOTE Confidence: 0.902594745159149

00:37:01.250 --> 00:37:02.020 What does it?

NOTE Confidence: 0.788671493530273

00:37:02.520 --> 00:37:08.010 TCR repertoire look like is that clonally expanded.

NOTE Confidence: 0.899585723876953

00:37:08.880 --> 00:37:11.080 Are they recognizing something else?

NOTE Confidence: 0.916913092136383

00:37:13.950 --> 00:37:21.230 Is a successful immune responses that comprise of a monolithic expansion of one super duper PCR?

NOTE Confidence: 0.901375532150269

00:37:21.930 --> 00:37:25.550 Or is it more abroad and maybe not so Super Duper.

NOTE Confidence: 0.947189450263977

00:37:30.580 --> 00:37:34.130 What are the contributions of the cervical vaginal microbiome?

NOTE Confidence: 0.861784160137177

00:37:34.740 --> 00:37:47.730 We now on these lanja tude. Inal's specimens able to characterize quantitate. The microbiome in the cervical cervix at these different time points course we're also looking for other.

NOTE Confidence: 0.898871719837189

00:37:48.380 --> 00:38:09.480 Pathogen since they live there, but is there something about the microbiome. We have a sense that in the cervix is backwards from everywhere else, having less heterogenous city is a good thing in the cervix, whereas if the microbiome broadens out that's a bad thing.

NOTE Confidence: 0.926619827747345

00:38:11.040 --> 00:38:15.950 Which is really good I can study the cervix microbiome because I really did not want to study poop?

NOTE Confidence: 0.893055975437164

00:38:17.630 --> 00:38:26.510 Does vaccination change this microbiome? I you know? I listen to these talks and I wonder? I have always had this chicken egg question.

NOTE Confidence: 0.925635635852814

00:38:29.650 --> 00:38:51.510 These are still relatively early days I forget who it was 2% right 2% of your memory. Cells are in circulation. The rest of them are tissue resident memory cells. Most of which are resident in non sterile barrier. Epithelium there each going to have different rules for immune homeostasis, most of which we don't know.

NOTE Confidence: 0.924142599105835

00:38:53.620 --> 00:39:00.690 But if you think about it if the rheostat is set a little bit, too fast. That's what an autoimmune disorder is so.

NOTE Confidence: 0.896133005619049

00:39:01.670 --> 00:39:08.690 You're going on a little bit Fast forward in the skin. That's like eczema or psoriasis and the gut IBD.

NOTE Confidence: 0.933051288127899

00:39:09.720 --> 00:39:11.780 In the central nervous system Ms.

NOTE Confidence: 0.695153653621674

00:39:14.980 --> 00:39:15.500 And.

NOTE Confidence: 0.926472306251526

00:39:17.710 --> 00:39:25.070 Why is the immunobiology of HPV cancer so radically different depending on that issue primary site?

NOTE Confidence: 0.918444097042084

00:39:25.700 --> 00:39:35.860 By that I mean, if a woman comes in with an HPV cancer cervical cancer and it's metastasized to lymph nodes. I have nothing to give her palliative care.

NOTE Confidence: 0.929475843906403

00:39:37.550 --> 00:39:41.020 Whereas if somebody comes in with a head and neck HPV cancer.

NOTE Confidence: 0.891576528549194

00:39:41.870 --> 00:39:47.760 If it's Stage 4 involving lymph nodes. We cure 80% of them? What is up with that right?

NOTE Confidence: 0.928127944469452

00:39:51.190 --> 00:40:06.170 I think we don't know enough about anal disease, but of course, here is an opportunity to look at outcomes between well. People who are living with well controlled HIV compared to people who are immune competent.

NOTE Confidence: 0.93667995929718

00:40:06.670 --> 00:40:08.410 Because it turns out of course now.

NOTE Confidence: 0.907691895961761

00:40:09.180 --> 00:40:26.330 People living with HIV are almost reconstituted their viral load goes and undetectable their CD 4 count comes up to normal. So these people are living reasonable lives and they're dying of malignancy's caused by viruses.

NOTE Confidence: 0.933536648750305

00:40:28.450 --> 00:40:30.060 So many questions we can ask.

NOTE Confidence: 0.761094033718109

00:40:30.920 --> 00:40:32.080 This is

NOTE Confidence: 0.891997456550598

00:40:32.920 --> 00:40:39.680 A diagram that Lawrence lead vocal published a really long time ago, but as she often is she's on target.

NOTE Confidence: 0.911071419715881

00:40:40.190 --> 00:40:57.180 What are the immunomodulatory effects of these conventional cancer treatment modalities knowing these will give us a sense of what in? What sequence to give therapy and there's some basic kinds, but I?

NOTE Confidence: 0.854450047016144

00:40:57.830 --> 00:41:13.600 Use these immune suppressive cells as an example because it's just really down to Earth, one way that MB SC function is they secrete IDO and arginase.



NOTE Confidence: 0.878585875034332

00:41:14.390 --> 00:41:34.220 Into the micro environment. Now it turns out there's one enzyme upstream of both of them called phosphodiesterase 5 and if that's blocked if that's blocked you don't get ID. Oh, an arginase downstream and of course, there's a commercially available.

NOTE Confidence: 0.903653681278229

00:41:35.290 --> 00:41:41.710 Blocker that we administer for prescribed for you could argue completely non malignant reasons.

NOTE Confidence: 0.810165047645569

00:41:42.320 --> 00:41:44.400 Some of your giggling do you know what it is?

NOTE Confidence: 0.604828417301178

00:41:45.190 --> 00:41:46.010 This Viagra.

NOTE Confidence: 0.877255618572235

00:41:46.640 --> 00:41:54.560 So Can you imagine doing a clinical trial with a 10 day run in of I Ghagra I mean, you'd accrue in about 2 weeks.

NOTE Confidence: 0.884856462478638

00:41:56.620 --> 00:42:12.320 And the hope is also that we can use these intelligent way. For example, platinum based therapy intercalates into the DNA and so you see damage and so you could envision a sequence that would involve.

NOTE Confidence: 0.890746355056763

00:42:13.940 --> 00:42:22.190 Giving sublethal chemo radiation in the case of HPV cancers that would consist of.

NOTE Confidence: 0.894120216369629

00:42:22.780 --> 00:42:36.140 Carbot axalan concurrent radiation and the idea is to create a very immunologically noisy cell death now event case in the leaves is reported that in patients who have been treated that way.

NOTE Confidence: 0.904475152492523

00:42:36.920 --> 00:42:50.170 The frequency of myeloid derived cells in their circulation goes way down and stays there for about 2 weeks. And so you could envision giving somebody a boost vaccination.

NOTE Confidence: 0.905539155006409

00:42:50.760 --> 00:42:59.130 Before or after actually but definitely to put the PD one blockade within that two week window a 2 month window.

NOTE Confidence: 0.945076406002045

00:43:03.920 --> 00:43:07.880 These methods can be applied to the study of any solid tumor.

NOTE Confidence: 0.911852180957794

00:43:08.880 --> 00:43:34.050 This is the basic outline figure out a signature in the target lesion that predicts outcome. We're not doing anything fancy. 'cause I don't have anything fancy so this is multi Plex for CD8 CD for PD. One PD L1. We have another 4 panel that includes CD 68, so you can get quantitative information about the intensity and Co localization of.

NOTE Confidence: 0.896531939506531

00:43:34.750 --> 00:43:43.020 Infiltration of different immune cells subsets in different compartments of the tumor and do it in a quantitative way.

NOTE Confidence: 0.888319194316864

00:43:43.770 --> 00:44:00.430 We are tissue specimens are really tightly clinically annotated and we know everything about these patients except for maybe what they had for breakfast. And this isn't great contrast to studies, which are important, but I've looked at 100 normal 100 high grade hung 100 cancer.

NOTE Confidence: 0.905607879161835

00:44:00.980 --> 00:44:04.640 Here we can see the evolution in a single patient.

NOTE Confidence: 0.88769543170929

00:44:05.930 --> 00:44:27.060 In this image analysis of course, we do in a quantitative way. The computer is not me. And we can look for as I said intensity of infiltration of different tumor compartments. We can look at the intensity spatial relationships and after that embark on doing.

NOTE Confidence: 0.835648238658905

00:44:28.080 --> 00:44:35.100 All the omcs so we use the histologic context to guide isolation of.

NOTE Confidence: 0.927132844924927

00:44:36.190 --> 00:44:41.000 Immune cells of choice for example, what's going on in a tumor that.

NOTE Confidence: 0.805901348590851

00:44:41.820 --> 00:44:43.660 Doesn't let CDA 2000?

NOTE Confidence: 0.875987589359283

00:44:44.250 --> 00:44:46.250 You don't know what's going on in the stroma don't you?

NOTE Confidence: 0.946260333061218

00:44:47.370 --> 00:44:49.020 So you can start looking at these.

NOTE Confidence: 0.905525326728821

00:44:49.690 --> 00:45:01.530 Different Oh mix and one thing you should have lodge in your brains so far. Today is that these tumors are incredibly heterogeneous and so the idea of.

NOTE Confidence: 0.890276193618774

00:45:02.080 --> 00:45:29.550 If you get a piece of tissue the size of a pencil eraser and you think Oh goodie. I've got tumor. You know there's a lot going on in that piece of tissue and you get Brazilians of pieces of data from these things and in my mind, I think of kind of is that is kind of throwing a ball of mud at the wall to see what sticks we can also do things like protein expression. There are epigenetic changes that we see in the stroma.

NOTE Confidence: 0.922588884830475

00:45:30.340 --> 00:45:41.910 If you figure out his signature in the tissue. That's diagnostic or prognostic or can be used to monitor the next question is can, we find the signature in the blood?

NOTE Confidence: 0.90554141998291

00:45:44.510 --> 00:46:16.420 Well, if we can find it in the blood could we find it in the urine now. This happens to be possible. You may be aware that the way we used to follow people who had a slightly elevated. PSA was they would have a slightly elevated PSA and you would do something rude to their rear end, nothing going on So what do you do you bring him back in 6 months and you get a blood and then you it's kind of the same and you bring him back in 6 months and it's kind of the same. The reason we follow these patients so closely is to catch the one guy who's disease.

NOTE Confidence: 0.811752796173096

00:46:16.420 --> 00:46:20.500 Takes off my colleagues Michael Donovan and his.

NOTE Confidence: 0.893220007419586

00:46:21.230 --> 00:46:37.850 Found this figured out his signature in the prostates that had taken off and then validate it in 200 or hundreds of prostatectomies. This signature was pretty simple. It was 3 different proteins. So they look for it and exosomes in the blood.

NOTE Confidence: 0.911733865737915

00:46:38.930 --> 00:46:43.310 And by Gosh it's in the urine and that test is now been FDA approved.

NOTE Confidence: 0.947402119636536

00:46:45.420 --> 00:46:51.920 If you can imagine being able to screen for HPV disease that way that would be actually.

NOTE Confidence: 0.893338859081268

00:46:52.440 --> 00:47:01.280 A game changer because outside of the cervix non cervical HPV cancers. We have no way yet to screen for them.

NOTE Confidence: 0.917956352233887

00:47:03.320 --> 00:47:06.120 OK, I'm going to finish up by leaving you with.

NOTE Confidence: 0.872145533561707

00:47:07.920 --> 00:47:09.650 Words of caution.

NOTE Confidence: 0.947023391723633

00:47:11.670 --> 00:47:16.730 We've gotten to the point where we think that that issue. What's going on in that issue is the truth.

NOTE Confidence: 0.862066268920898

00:47:18.170 --> 00:47:26.220 Here are the Top 15 most frequent V Baiters in patient frozen tissue from her re section.

NOTE Confidence: 0.884892106056213

00:47:28.930 --> 00:47:34.330 OK, if you do it, the way everybody else does it and does dis aggregation?

NOTE Confidence: 0.839785039424896

00:47:35.180 --> 00:47:37.230 It looks for the same view betas.

NOTE Confidence: 0.940828740596771

00:47:38.380 --> 00:47:46.730 You can see that what you're studying is what survived is not necessarily what was actually going on in a tissue.

NOTE Confidence: 0.906006991863251

00:47:47.890 --> 00:47:52.980 And then just for the sake of comparison here at the frequencies of those feed betas in the blood.

NOTE Confidence: 0.84399551153183

00:47:54.330 --> 00:47:55.950 It's true in mice, too.

NOTE Confidence: 0.873094022274017

00:47:57.480 --> 00:48:08.230 Desegregated tumors David Masopust published a paper in which he looked at frequencies in cells now then he estimated.

NOTE Confidence: 0.90028965473175

00:48:08.810 --> 00:48:16.050 The numbers of T cells and 5 different mouse organs 5 different mouse organs in two ways that were completely quantitative.

NOTE Confidence: 0.890105783939362

00:48:16.800 --> 00:48:27.200 One way he did serially sectioned organs and then staying for CD3 and then extrapolated to get a sense for how many cells for organ there were.

NOTE Confidence: 0.862456679344177

00:48:27.720 --> 00:48:32.070 And another approach he?

NOTE Confidence: 0.836498856544495

00:48:32.960 --> 00:48:41.100 Got whole tumor DNA and figured out the number of cells for organ that way and they correlated pretty tightly.

NOTE Confidence: 0.859778702259064

00:48:42.190 --> 00:48:43.890 Oh here's the kicker.

NOTE Confidence: 0.887009382247925

00:48:44.700 --> 00:49:06.550 When he did you know dis aggregation and saw what came out the black bars are? What you got when you did, it this way, and the Gray bars or what came out from the tumor infiltrating lymphocytes basically if your organ was kind of squishy the more likely you are you are to recover about the same number.

NOTE Confidence: 0.917350590229034

00:49:07.440 --> 00:49:16.920 In the female reproductive tract that was so not the case as you can see here and part of the problem also this scale is a log scale.

NOTE Confidence: 0.934185743331909

00:49:19.960 --> 00:49:26.550 So the problems with this approach is that you get really just a fraction of that issue immune cells.

NOTE Confidence: 0.918237447738647

00:49:27.640 --> 00:49:36.750 Unless you have another chunk of tissue from the same adjacent to where you did. This you have lost the histologic context. So you don't know what's going on?

NOTE Confidence: 0.903164386749268

00:49:38.060 --> 00:49:50.720 And, of course, physical manipulation activate cells and it just explodes macrophages. So what are pathologists easing methodological approach in grant application or a paper? We call it grinding find?

NOTE Confidence: 0.928464293479919

00:49:51.820 --> 00:49:54.450 And really the thing I want to leave you with is that?

NOTE Confidence: 0.941438555717468

00:49:55.480 --> 00:50:06.570 If you're studying immune responses using this approach, you should be aware that you may be asking a very different question from the question you think you're asking.

NOTE Confidence: 0.760450780391693

00:50:09.450 --> 00:50:11.990 OK NBC people laugh at this.

NOTE Confidence: 0.933698713779449

00:50:14.440 --> 00:50:28.250 Well, how the heck do you do that? How do you get unmanipulated cells from a specific micro environment and figure out what they're doing because a lot of the single cell sequencing requires that you have viable single cell.

NOTE Confidence: 0.912515997886658

00:50:28.700 --> 00:50:41.460 Solutions that doesn't happen in solid tumors, so far, so we developed this approach. We have figured out how to do rapid immunostaining in under 30 minutes on Cryo sections.

NOTE Confidence: 0.909755706787109

00:50:42.010 --> 00:50:50.080 And then the post doc runs downstairs. We've this is pattern recognition recognition software. I want you to look.

NOTE Confidence: 0.903108060359955

00:50:51.300 --> 00:51:06.270 Right now keep your I'm right there. Did you see how instantaneous that was we ask the machine to go? Find everything this? Is the lower level of Brown. This is the upper level. It's just like thresholding for flow go. Find everything that's at least this big but smaller than that.

NOTE Confidence: 0.860222518444061

00:51:07.380 --> 00:51:12.570 And So what Leo is doing now is cleaning it up because we didn't threshold it quite right.

NOTE Confidence: 0.9244225025177

00:51:14.570 --> 00:51:19.660 Conversely, if you think it missed something you can just circle, it on the screen because it's a bamboo screen.

NOTE Confidence: 0.88976514339447

00:51:20.650 --> 00:51:21.970 And you can see we're getting a.

NOTE Confidence: 0.901151835918427

00:51:22.580 --> 00:51:23.300 Spreadsheet.

NOTE Confidence: 0.808314561843872

00:51:25.080 --> 00:51:27.500 Here of the absolute area OK.

NOTE Confidence: 0.810098171234131

00:51:28.730 --> 00:51:29.490 What happens?

NOTE Confidence: 0.847044765949249

00:51:34.430 --> 00:51:37.470 These are half bars sells these are placental macrophages.

NOTE Confidence: 0.881952404975891

00:51:38.080 --> 00:51:39.690 These are unmanipulated.

NOTE Confidence: 0.860504388809204

00:51:40.550 --> 00:51:51.490 Specific cell subsets from a known histologic context. And so you can then go on and do omics within the lifetime over post stuff.

NOTE Confidence: 0.903410375118256

00:51:53.690 --> 00:52:17.900 I'm going to rap with this slide. This is a table of the methods that we've developed using human tissue specimens, which are all Eddy bitty because of course, the first priority is to make sure not compromise. The ability to make an accurate diagnosis. But we these methods are of course, evolving we can do more and more with the FFPE.

NOTE Confidence: 0.942053973674774

00:52:20.240 --> 00:52:24.250 So that's what I got thank you for your attention and I'd be glad to take questions.

NOTE Confidence: 0.671710073947906

00:52:33.930 --> 00:52:37.460 We all believe Maine.

NOTE Confidence: 0.893265306949615

00:52:40.990 --> 00:53:00.600 Have you started to look at any of the with the viral antigens. The possibility exists that as with the vaccines that you could have off the shelf products that might work for some patients or the idea of using those shared or common antigens across patients any ideas related to that.

NOTE Confidence: 0.87457674741745

00:53:01.870 --> 00:53:21.890 Aside from the vaccines, obviously one of her yeah, yeah, yeah, I mean, ideally in terms of vaccine, yeah, but also in terms of TC, Rs you could imagine somebody comes in has a HPV disease and you get there. HLA profiling and say, Oh, we have a really good T cell receptor for HLA 8, two and a really good one for.

NOTE Confidence: 0.907446920871735

00:53:22.440 --> 00:53:25.300 So and so and those would be off the shelf.

NOTE Confidence: 0.892622649669647

00:53:27.590 --> 00:53:48.380 There's really interesting I'm wondering about the basement membrane and why it is cells kind of sit there any thoughts? Yeah, one reason is because the neo vasculature that we look for in these lesions that has downregulated expression of the adhesion molecules, which is why we scared up.

NOTE Confidence: 0.7496018409729

00:53:48.950 --> 00:53:50.820 Innate response.

NOTE Confidence: 0.860337734222412

00:53:51.380 --> 00:54:21.430 With the with the Aldara to activate that endothelium, but there are other things going on without it out there in that issue right there. Those cells are in that issue. So why do they just stop at the basement membrane they can't degrade it or not? That's a very complicated. Question part of it relates to what Greg talked about this morning. There's not a lot of their starved right there on purpose.

NOTE Confidence: 0.893387615680695

00:54:21.430 --> 00:54:43.810 Of course, the lesions even at this point have recruited immune suppressive subsets. There are many reasons and I'd be happy to work with anybody on these yeah, and now that we can actually quantitate the microbiome. Most of the studies that I've seen her like counting Jelly beans and it's good to know that this.

NOTE Confidence: 0.924391686916351

00:54:44.830 --> 00:54:54.350 Clinical setting is associated with this kind of diversity in this kind of pH and this clinical setting is associated with what but now we have.

NOTE Confidence: 0.920413613319397

00:54:55.370 --> 00:55:12.860 The opportunity to quantitate the microbiome and know what's going on in that issue at the same time, So what is it may be about the quality of how the microbiome activates a local innate response that contributes to this cold thing and vice versa.

NOTE Confidence: 0.873835206031799

00:55:16.600 --> 00:55:44.230 Of the Treasury Invoice structure in the responders. It's looks great, so have you thought about whether the B. Cells are T cells in the Treasury. Linvoy structure recognize the same type of antigen as that's in the lymph node question is what are the cells in the tertiary lymphoid structures recognizing we're working on it right now with Kelly Smith with the manifest technique So what we do is.

NOTE Confidence: 0.881587445735931

00:55:45.070 --> 00:56:15.400 You can either predict epitopes or just overlapping pep ties its cover E 687 and take the fissions peripheral blood and put one



peptide per well and you can pick out the ones that get activated and so you know, those are recognizing some piece of the HPV so we then go and look in that issue to see if we see those 2 CRS is very difficult to measure function from.

NOTE Confidence: 0.902001321315765

00:56:15.400 --> 00:56:21.920 2 cells that you pulled out of tissue it's almost if they need something in the tissue to be activated.

NOTE Confidence: 0.844975709915161

00:56:23.600 --> 00:56:45.760 More questions so right now there a vaccination against HPV so for those people are vaccinated, with the HPV vaccine with Ivy like saying are they are the bees are repertoire in those patients similar to the patients with this vaccine, you in the clean code file.

NOTE Confidence: 0.902539014816284

00:56:46.270 --> 00:56:51.280 I don't know because I'm a 2 cell chauvinist, but I'd be happy to look at B cell responses with you.

NOTE Confidence: 0.931680381298065

00:56:54.880 --> 00:56:55.290 Thank you.