Everyone we’re going to get started if so, if anyone is out 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.

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.

Good afternoon and thank you in advance for your attention.

And, of course for staying awake right after lunch.

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 Anecdotally 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: 0.711628854274775

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

NOTE Confidence: 0.865569877791024
In the setting of a persistent HPV infection.

You get soon 3 from 40,000 feet. You can see that in these cells 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.

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.

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.

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.

In this setting.

Of a persistence in 3, I should not able to show you this last column in this day and age.

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.

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

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

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 resection 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

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: 0.90455485427856

And then had a post up hundreds of women actually participated in this study an were very, very grateful to them because we learn some very useful things first of all.

NOTE Confidence: 0.912821412086487

We compare the diagnostic biopsy to the Week 15 tissue resection 20% of these things were gone.

NOTE Confidence: 0.865731656551361

At Week 15.

NOTE Confidence: 0.870663523674011

How does that happen?

NOTE Confidence: 0.887655138969421

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?
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.

HPV specific immune responses.

In the serom which we also have lanja tude only we can look for endosomes and cell free DNA.

On the frozen tissue, which I also get when I do the reception, which they can do because I'm a surgeon.

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.

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.

This is not a sterile environment.

What do we learn OK no lesions progressed during the study window?

In some patients endogenous mechanisms could eliminate incipient cancers.

OK light bulb moment.
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.

And permitted that to persist.

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.

So Drew and TC, who had made the DNA vaccine, which was.

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.

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.

This is one of those, 1 slide 3 years of work slides so anyway. The problem was nothing happened.

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.
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.

Well. 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.

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.

Nothing happened so these are immune responses to E 7 in patients who got.

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.

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.

OK, what was the lesson there, both need to find a vaccine strategy that immunogenic in people.

And at that point I decided to put on my pathology hat.

And you can see right here. You don’t need to be a pathologist to see where the party is.
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. Downreg-
ulates 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
And in contrast, here’s a lesion that.

Kept them out and this is the expression of mad cow.

We had to quantitate this you couldn’t to see right in any case, we did. We developed pattern recognition software.

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 an in CIN epithelium and stroma. The tightest correlation was in CIN epithelium with the correlation was actually .88.

Would that tell us if we made a therapeutic re- sponse, probably we should think about ways to activate the vascular endothe- lium 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.

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.

We actually found a vaccine that made an immune response in humans. It’s housed in a Recon vaccinia vector.

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.

And away we go.
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.

And.

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 exf evil tinkering.

That’s a really hard step actually. But we had a runner on base.

Now then we wanted to know of course, whether tinkering with the micro environment directly could improve the efficacy of therapeutic vaccine vaccination.

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 basil 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.

Innate response wherever you put it now, then all of these patients had HPV 16 associated disease.

It turns out that HPV 16 associate is associated with about 60% of high grade lesion So what does that mean?

That means 40% are associated with other Geno Types and I had all these patients crying in my office because they nickel MoD is.
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.

Now then the last group got both the highest dose of vaccination as well as imiquimod applied directly to the cervix.

We saw something so striking.

In these vaccinated, patients that we published these results halfway through the trial actually So what I’m going to show you is.

Dinner from these patients who got only vaccination Noah Meelman and I’ve put on the Top right corner expected rate of spontaneous regression just in case you forgot it.

One big question in the field with.

Can peripheral vaccination make an immune response?

That goes we’re supposed to.

And we think so.

I’m going to show you subject matched tissue specimens from before and after vaccination.

Here’s one patience lesion before vaccination.

Here’s after.
Here’s Tibet before.

So their killers.

This is a slide this next set just.

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.

The only reason that a T cell or at least the T op five reason that a T cell starts for live rating is if it’s been activated by its cognate antigen.

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.

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.

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?

You want to get really the reason it.
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.

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.

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

00:21:39.960 --> 00:22:04.390 So there’s no way I could look at 12 of these things and not put together the same story in my head.

00:22:05.060 --> 00:22:07.630 It turned out that the frequencies of Clonally expanded debaters.

00:22:09.610 --> 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.

00:22:05.610 --> 00:22:07.630 This is within subject we did this with adaptive.

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.

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.

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

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.
I don’t know what that means there’s 55 and they drop out. It’s so clean.

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.

None of this is conclusive it’s all indirect. But overall, it does suggest that T cells in the tissue were there by a process of selection and not by leakage.

Functional phenotype, so there, I go sitting in the dark again did laser capture microdissection of the same cases.

And looked at the stroma subjacent immediately to residual CIN.

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.

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.

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.

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

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

00:24:41.610 --> 00:25:05.720 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.

00:25:06.240 --> 00:25:11.690 From low power this is her residual disease, I think it’s.

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

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

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.

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

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

00:24:56.240 --> 00:25:00.770 From low power this is her residual disease, I think it’s.
So what did we learn OK. These were all important things to know crucial vaccination can in fact change.

The immune context in the target lesion, I know where to go.

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.

And it also in these patients technically the relations didn’t go completely away. So what we realized is that.

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.

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?

Kiss of death.

What do shrinky things look like it’s kind of?

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.

Kiss of death.
We cured.

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.

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.

Here we have.

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.

And the phase 3 is now on going.

Well, that was really fast overview of.

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.

Don’t be discouraged.

This is something completely new and different I mean, I was a T cell chauvinist because I was raised by that guy, but then.
We happened upon this.

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.

And can’t keep food down, we give it to him up there little bumps in a Suppository.

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.

OK.

So it turns out that art estimate is cytotoxic to many human solid tumor cell lines.

In a free clinical dog model this is Dick Schlegel’s group.

We don’t exactly totally know why.

Of course, it was decided toxic to cervical cancer cell lines and it turned out, it was also cytotoxic.

In a free clinical dog model this is Dick Schlegel’s group.

Here’s a United papilloma virus.

Lesion treated with the active form of art estimate. These are just like casing beliefs evolve our pictures, it’s gone.
Our first clinical trial of Artesa Nate was.

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 2 and 4.

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.

The first treatment group was a low dose 50 milligrams.

The next treatment group was the full dose. We planned to give one cycle each cycle is 5 consecutive nights.

The second, the 3rd treatment group got the same dose but 2 cycles.

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.

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.
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.

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.

Maybe all of us have this patient.

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.

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.

It’s been more than a year now, and she has still no detectable HPV.

And she had been dealing with this repetitive high grade for 15 years well.

It’s been more than a year now, and she has still no detectable HPV.

So we gave it to a second patient amended the protocol gave it to a second patient.
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

OK, that’s what’s new what’s next.

NOTE Confidence: 0.947767078876495

What does a successful immune response look like?

NOTE Confidence: 0.915941119194031

We’re very fortunate position to have a lesion.

NOTE Confidence: 0.894746661186218

And no histologic context and know a lot of other things, from the same time points.

NOTE Confidence: 0.91115266084671

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

Now before vaccination at times 0.

NOTE Confidence: 0.819451928138733

This is what her biopsy look like that’s cold like in England.

NOTE Confidence: 0.918851613998413

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

She did this.

NOTE Confidence: 0.904788851737976

So we can ask begin to ask questions now about.

NOTE Confidence: 0.921139359474182

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

So being
A party here. 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 Apple ptosis, but those are all indirect. 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. 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, Rs tissue. In something that looks like that. What does it? TCR repertoire look like is that clonally expanded. Are they recognizing something else? Is a successful immune responses that comprise of a monolithic expansion of one super duper PCR? Or is it more abroad and maybe not so Super Duper. What are the contributions of the cervical vaginal microbiome?
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.

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.

Which is really good I can study the cervix microbiome because I really did not want to study poop?

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.

But if you think about it if the rheostat is set a little bit, too fast. That’s what an autoimmune disorder is so.

You’re going on a little bit Fast forward in the skin. That’s like eczema or psoriasis and the gut IBD.

In the central nervous system Ms.

Why is the immunobiology of HPV cancer so radically different depending on that issue primary site?
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.

Whereas if somebody comes in with a head and neck HPV cancer.

If it’s Stage 4 involving lymph nodes. We cure 80% of them? What is up with that right?

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.

Because it turns out now 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.

So many questions we can ask.

A diagram that Lawrence lead vocal published a really long time ago, but as she often is she’s on target.

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?
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.

Blocker that we administer for prescribed for you could argue completely non malignant reasons.

Some of your giggling do you know what it is? This Viagra.

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.

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.

Giving sublethal chemo radiation in the case of HPV cancers that would consist of.

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.

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.

Before or after actually but definitely to put the PD one blockade within that two week window a 2 month window.
These methods can be applied to the study of any solid tumor.

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.

Infiltration of different immune cells subsets in different compartments of the tumor and do it in a quantitative way.

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.

Here we can see the evolution in a single patient.

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.

Immune cells of choice for example, what’s going on in a tumor that.

All the omcs so we use the histologic context to guide isolation of.

Immune cells of choice for example, what’s going on in a tumor that.

Doesn’t let CDA 2000?

You don’t know what’s going on in the stroma don’t you?

So you can start looking at these.
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.

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.

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?

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.

Takes off my colleagues Michael Donovan and his. 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.

And by Gosh it’s in the urine and that test is now been FDA approved.

If you can imagine being able to screen for HPV disease that way that would be actually.
A game changer because outside of the cervix non-cervical HPV cancers. We have no way yet to screen for them.

OK, I’m going to finish up by leaving you with words of caution.

We’ve gotten to the point where we think that that issue. What’s going on in that issue is the truth.

Here are the Top 15 most frequent V Baiters in patient frozen tissue from her resection.

OK, if you do it, the way everybody else does it and does disaggregation?

You can see that what you’re studying is what survived is not necessarily what was actually going on in a tissue.

And then just for the sake of comparison here at the frequencies of those feed betas in the blood.

It looks for the same view betas.

You can see that what you’re studying is what survived is not necessarily what was actually going on in a tissue.

And then just for the sake of comparison here at the frequencies of those feed betas in the blood.

It’s true in mice, too.

Desegregated tumors David Masopust published a paper in which he looked at frequencies in cells now then he estimated.

The numbers of T cells and 5 different mouse organs 5 different mouse organs in two ways that were completely quantitative.
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.

And another approach he?

Got whole tumor DNA and figured out the number of cells for organ that way and they correlated pretty tightly.

Oh here’s the kicker.

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 are you are to recover about the same number.

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.

So the problems with this approach is that you get really just a fraction of that issue immune cells.

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?

And, of course, physical manipulation activate cells and it just explodes macrophages. So what are pathologists eases methodological approach in grant application or a paper? We call it grinding find?

And really the thing I want to leave you with is that?
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.

OK NBC people laugh at this.

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.

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.

And then the post doc runs downstairs. We’ve this is pattern recognition recognition software. I want you to look.

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.

And So what Leo is doing now is cleaning it up because we didn’t threshold it quite right.

Conversely, if you think it missed something you can just circle, it on the screen because it’s a bamboo screen.

And you can see we’re getting a.

Spreadsheet.

Here of the absolute area OK.
What happens?

These are half bars sells these are placental macrophages.

These are unmanipulated.

Specific cell subsets from a known histologic context. And so you can then go on and do omics within the lifetime over post stuff.

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.

So that’s what I got thank you for your attention and I’d be glad to take questions.

We all believe Maine.

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.

Aside from the vaccines, obviously one of her 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.
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

Innate response.

NOTE Confidence: 0.860337734222412

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

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

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

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

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

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.