Good afternoon, my name is Katie Politi and I'm an associate professor of pathology and medicine here at the Yale School of Medicine and Awesome Co. Leader of the cancer Signaling Networks. It is my pleasure to introduce Doctor Michael Shen today. Doctor Shen is a professor of medicine, genetics and development, urology and systems biology at Columbia University Medical Center. He received his undergraduate
degree from Harvard University and his PhD from Cambridge University. He then pursued his postdoctoral training with Phil, Doctor Phil, leader at Harvard Medical School, before becoming an independent investigator at Rutgers in 1994 and moved to Columbia University Medical Center in 2007. He is currently the Co leader of the Tumor Biology and Micro Environment Program at the Herbert Irving Comprehensive Cancer Center, as well as the director of Graduate studies in the Columbia Department of Genetics and Development.
Over the past 26 years, Doctor Shen has investigated fundamental mechanisms of mammalian development in cancer using in vivo analysis of genetically engineered mouse models. Recently his lab has generated novel culture conditions for mouse and human, prostate and bladder organized noise, which have led to the creation of a biobank of patient arrived bladder tumor organoids. Current work in the lab focuses on understanding molecular mechanisms of cell type differentiation in the normal as well as the transformed prostate epithelium. The epigenetic regulation of linear plasticity in both...
bladder and prostate cancer, and the role of the tumor micro environment in modulating treatment response. Doctor Sen, it really is a pleasure to have you here today and have you visit. I’ll be at virtually from Columbia University, so thank you very much and we look forward to your presentation. Well, thank you Katie. It’s a real pleasure to have this opportunity to speak to. This audience that yell and I wish this were in person, but.
00:02:38.260 --> 00:02:40.416 Make do as best as we can,
NOTE Confidence: 0.9015736
00:02:40.420 --> 00:02:42.330 so I’ll go ahead and
NOTE Confidence: 0.9015736
00:02:42.330 --> 00:02:46.610 share my screen. Um? And.
NOTE Confidence: 0.9336113
00:02:50.290 --> 00:02:54.410 Hopefully. You can see my presentation.
NOTE Confidence: 0.9336113
00:02:54.410 --> 00:02:57.868 Is that true? Can everyone see my?
NOTE Confidence: 0.9336113
00:02:57.868 --> 00:02:59.708 Yes we can see it.
NOTE Confidence: 0.9336113
00:02:59.708 --> 00:03:01.180 Thank you. Yes excellent.
NOTE Confidence: 0.9336113
00:03:01.180 --> 00:03:03.388 So today I’d like to talk.
NOTE Confidence: 0.9336113
00:03:03.390 --> 00:03:05.598 Take the opportunity to discuss published
NOTE Confidence: 0.9336113
00:03:05.598 --> 00:03:08.754 work, but also a lot of work that can
NOTE Confidence: 0.9336113
00:03:08.754 --> 00:03:11.119 be construed as work in progress.
NOTE Confidence: 0.9336113
00:03:11.120 --> 00:03:13.730 Much of it focusing on the
NOTE Confidence: 0.9336113
00:03:13.730 --> 00:03:15.470 issue of linic plasticity.
NOTE Confidence: 0.9336113
00:03:15.470 --> 00:03:18.116 And we’ve been studying this through
NOTE Confidence: 0.9336113
00:03:18.116 --> 00:03:20.989 in vivo analysis in mouse models,
NOTE Confidence: 0.9336113
00:03:20.990 --> 00:03:23.290 but also using organoid models,
and we've been studying this in both the prostate and the bladder. So to start with, what is plasticity? So if we consider that plasticity in the most general definition is the ability of a cell to change from one identity to another, we can think of this in the Waddington model. You know, perhaps cliched Waddington model. As you know, sort of balls rolling down a Hill. We start with a stem cell and we have various differentiated cell types and the ability of cells too. Basically change their identity.
00:04:01.620 --> 00:04:04.020 is considered to be plasticity
NOTE Confidence: 0.9336113
00:04:04.020 --> 00:04:06.790 and so there are different forms
NOTE Confidence: 0.9336113
00:04:06.790 --> 00:04:09.005 there sort of reprogramming back
NOTE Confidence: 0.9336113
00:04:09.086 --> 00:04:11.046 to a more progenitor state.
NOTE Confidence: 0.9336113
00:04:11.050 --> 00:04:13.380 There can be transdifferentiation changing
NOTE Confidence: 0.9336113
00:04:13.380 --> 00:04:16.040 from one identity to another etc.
NOTE Confidence: 0.9336113
00:04:16.040 --> 00:04:19.480 So this is a process that has been
NOTE Confidence: 0.9336113
00:04:19.480 --> 00:04:21.804 studied extensively in both development
NOTE Confidence: 0.9336113
00:04:21.804 --> 00:04:24.570 and cancer and it’s important to
NOTE Confidence: 0.9336113
00:04:24.570 --> 00:04:27.390 think about when we talk about
NOTE Confidence: 0.9336113
00:04:27.390 --> 00:04:30.158 plasticity in cancer to consider that.
NOTE Confidence: 0.9336113
00:04:30.158 --> 00:04:32.648 In order to study plasticity,
NOTE Confidence: 0.9336113
00:04:32.650 --> 00:04:35.878 it’s also essential to understand the
NOTE Confidence: 0.9336113
00:04:35.878 --> 00:04:38.030 normal pathways of differentiation
NOTE Confidence: 0.9336113
00:04:38.109 --> 00:04:40.527 so that one can distinguish what
NOTE Confidence: 0.9336113
00:04:40.527 --> 00:04:43.051 happens in the normal context from
what might happen in a tumor context. So over many years we’ve been studying these issues in the prostate, and more recently in the bladder, and to start with, I just like to consider, a basic review of the prostate in the mouse. The prostate has a distinct anatomy there. It sort of has lobular structure. There are four different lobes in the mouse. There’s the anterior prostate, the dorsal prostate, the lateral prostate, as well as the ventral prostate. However, in the human there is
something a little bit different.
The human prostate does not have
Instead, it can be distinguished at
the pathological level as having you know,
so the distinct anatomy of the
mouse and human prostate has been.
Of note,
for many years and has been used sort
The inability perhaps of the mouse to truly model human prostate cancer. Now we’ve known from studies over many years that there are many conserved signaling pathways in the like, but the relationship between the mouse and human prostate still remains somewhat mysterious, both in terms of normal development as well as the cell types can contain. Within the prostate. No. The prostate in the mouse is formed at late stages of fetal development and organic Genesis primarily takes place at neonatal stages.
The prostate form, through a process of ductal budding budding from the urogenital sinus and initial prostate buds are marked by expression of the homeo box. Jinan kicks 3.1 here, visualized by Beta Galactoside. ASA Valax Enoch in that we made a number of years ago and you can see even early on at the time of birth. Their buds that correspond to distinct lobes and at least initially and kicks 3.1, is expressed by all of the epithelial cells in the prostate. Prostate formation of course requires androgen signaling,
but the requirements for androgen receptor are actually fairly complex and they involve epithelial mesenchymal interactions. So androgen receptor is actually required in the urogenital mesenchyme for prostate formation. So if you delete androgen receptor in the urogenital mesenchyme here in a TFM una testicular feminize mutant. And you perform a tissue recombination assay as was first shown through. The studies of Jerry Kunia about four decades ago now the prostate will not form. However, if you delete androgen receptor in the
epithelium now you will form a prostate.

However, the prostate is not entirely normal.

For example, it lacks secretory protein production.

So what are the cell types that are found in the normal adult prostate?

Well in both the mouse and the human there’s an array of different cell types in both the epithelium and in the stroma that are just now really being able of being characterized in some detail, but historically we’ve considered the epithelium as containing three basic cell types.

There are the luminal cells which are the tall columnar secretory cells.
That produce the the prostate secretions.

There’s an underlying layer of basal cells.

These cells are androgen receptor low or negative, and express basal cytokeratins unlike the luminal cells,

which are are high and expressed lumenal cytokeratins.

And then there is a rare third type known as neuroendocrine cells.

These have been very understudied and I’ll touch upon these later in my talk.

So this is sort of the way we thought many years now, and classically,
brazil cells have always considered to be more interesting.

They appear to have more stemlike properties or does luminal cells have been considered to be somewhat boring,

but I think you know for many years we’ve thought that luminal cells are actually the interesting cells.

And now with the advent of single cell analysis,

we see that there’s considerable complexity.

In the luminal population.

So what do basil cells do?

Well, we believe that there is sort of.

A conserved ancestral role for basil cells and that’s in wound repair.
So here, for example, in the prostate. If we damage the luminal cells by deletion of idcat here in inducible and the epithelial cells will Slough off. And undergo a notice and the basil cells will actually differentiate into luminal cells to replace the loss luminal cells. So this is shown in cartoon form here. So there’s sort of a basil to luminal differentiation that takes place, so we think that this is a conserved function of basil cells in many tissues, and more recently.
work from Cedric Web Con Slab has demonstrated that is in fact the case. What about luminal cells? Well, luminal cells, as I mentioned, have been considered to be somewhat boring, but in fact, in ex vivo assays at least one could see that there are luminal progenitors that are by potent. So if we perform lineages marking of luminal cells and generate organoids that are wholly composed wholly derived from luminal cells, then we can see that there are basil cells that can be formed in these organoids, and these basil cells are marked.
Indicating their lumenal origin.

So of course this is an ex vivo assay and ex vivo cells often display more plasticity than is found in during normal development, and certainly in.

Normal context luminal cells are usually impotent, but we think that normal developmental processes, among other things or, can be constrained by the micro environment and ex vivo assays such as organoid formation can reveal developmental potential that can...
So for example, we think that the ability of luminal cells to display potency to be able to generate basil cells is actually an ability that occurs early in organic Genesis. So this is sort of a linear hierarchy as is currently understood, in which there are by potent basil progenitors generating both basal and luminal cells during organic Genesis.

But. Luminal progenitors were generally thought of unipotent, but in recent studies we found that in
00:12:16.680 --> 00:12:20.347 fact there is a BI potent luminal progenitor,
00:12:20.350 --> 00:12:24.095 so if we lineages mark luminal cells.
00:12:24.100 --> 00:12:26.340 At early postnatal stages
00:12:26.340 --> 00:12:29.700 using an inducible CK 8 driver,
00:12:29.700 --> 00:12:33.402 we can then mark luminal cells
00:12:33.402 --> 00:12:35.870 and then analyzed later.
00:12:35.870 --> 00:12:39.030 Luminal cells are marked,
00:12:39.030 --> 00:12:41.364 but also basil cells,
00:12:41.364 --> 00:12:43.770 and it's a reasonable fraction
00:12:43.770 --> 00:12:45.750 of basal cells that are marked,
00:12:45.750 --> 00:12:47.326 is fairly short lived.
00:12:47.326 --> 00:12:48.508 It's fairly transient,
00:12:48.510 --> 00:12:51.135 but it can still be detected at
00:12:51.135 --> 00:12:53.694 a week after birth, but again,
the ability of this by potent progenitor

NOTE Confidence: 0.8089924

quickly disappears thereafter.

NOTE Confidence: 0.8089924

So interesting, Lee,

NOTE Confidence: 0.8089924

both the by potent basil progenitor and

NOTE Confidence: 0.8089924

this by potent luminal progenitor do

NOTE Confidence: 0.8089924

not require androgen receptor function,

NOTE Confidence: 0.8089924

so if we delete androgen receptor.

NOTE Confidence: 0.8089924

In basil cells,

NOTE Confidence: 0.8089924

we find that there’s no effect on

NOTE Confidence: 0.8089924

the formation of luminal cells,

NOTE Confidence: 0.8089924

and similarly if we delete a

NOTE Confidence: 0.8089924

are in the luminal cells,

NOTE Confidence: 0.8089924

we don’t see any effect on the

NOTE Confidence: 0.8089924

generation of basil cells.

NOTE Confidence: 0.8089924

So in cartoon form then what we

NOTE Confidence: 0.8089924

think is going on is that there is
a urogenital epithelial progenitor that gives rise to both basal and luminal progenitors, initially the basal progenitor can be potent as well as the luminal progenitor. But that this potency is fairly transient, and then in adulthood both luminal and basal progenitors are generally unipotent. However, this period of potency is actually occurring in the first four weeks after birth, and interestingly, this is a time when androgen levels are very low.
At these pre pubertal stages, now there is one other interesting aspect of luminal cells, which is that in a series of studies we’ve shown that they are favored as cells of origin for prostate cancer, so we’ve shown this in a number of different transgenic mouse models, as well as a hormonal carcinogenesis model. If we mark basal cells in these models, for example in the hymec model or a tramp model which are well characterized, the basal cells are marked. But they don’t really contribute to tumors. However, we mark luminal cells now they...
00:14:50.098 --> 00:14:52.560 readily contribute to tumor formation.

So. What we think is going on then is that if we mark luminal cells,

they will contribute tumors,

and this argues that luminal cells are a cell of origin for prostate cancer.

If we mark Basil cells,

they don’t contribute to tumors.

However, if you explant these cells,

they will.

You know in this sort of ex vivo context.

For example, in a graft undergo a basil to luminal differentiation,

and now they can form tumors.

So we consider basal cells to be a
celeb mutation, whereas luminal cells. Are the true cell of origin. And again, we've analyzed this in multiple mouse models of prostate cancer. So because we've observed the same result every time, we think that luminal cells are generally favored as a cell of origin. So we now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin. We now have this view that luminal cells are generally favored as a cell of origin.
dissect the mouse prostate properly.

You might think that’s a bit of an over some bug, an exaggeration, considering we’ve been studying the prostate for over 20 years, but in fact it’s really not a trivial matter of two.

Dissect the individual mouse lobes all the way down to their junction with the urethra.

So this is sort of a view of sort of how we can dissect the weight mouse lobes.

This is actually in a green mouse, so you can see the lobes that are dissected.

And then we subjected these two
single cell RNA sequencing both
the whole prostate as well as
the individual lobes to analyze
we collaborated with
Roll Rabadan’s lab Raul has.
Developed you know,
amazing algorithms that are based upon
rather sophisticated mathematical
approaches for analyzing single cell data.
So in particular,
his laboratory has developed approaches
based upon random matrix theory,
which demonstrate that these
large arrays of data,
for example as one generates using so
single cell RNA sequencing are mostly noise,
so. If you consider. These as giant matrices, well in fact the distribution of eigenvalues follows a sort of conserved mathematical distribution known as the Marchenko Pastur distribution, and the deviation for this distribution is actually where the signal is, and typically it’s only about 2 to 3% of the data. So this is a hypothetical distribution. This is actually real data. This is one of our prostate datasets. And again, here is the signal,
so his laboratory is developed randomly,

an algorithm to isolate these data.

And this proved to be very useful in our analysis,

because it allowed us to identify a cell population that would have been very difficult to identify other ways.

So when we examine an aggregated data set of whole prostate, we observe. Five different luminal populations.

First of all, we only identify one Basil population. So basil cells are actually not heterogeneous,

but instead there’s heterogeneity in the luminal population.
So, first of all, there’s four distinct lumenal populations that correspond to the that are located distally in each distally in the anterior prostate distally in the distal dorsal prostate distally in the lateral movie and eventual. Then there is a population that we call Lumpi for proxamol. It is similar in all four lobes and it is found more approximately. Finally, there is a population that we call Paraurethral.
This population has both basal and luminal properties, and it is found in the region most adjacent to the urethra. So these are the distinct epithelial properties that we’ve identified. We’ve also identified heterogeneity in the stroma, but I won’t speak to that further. So, as you can note from this sort of illustration, there is diversity along the proximal distal axis. So to give you an idea of what we mean by proximal and distal axis,
00:19:37.150 --> 00:19:38.935 here’s an anterior prostate that’s been sort of splayed out and cut in histological section when we refer to distal, we’re actually referring to this whole region here. That is more than 90% of the volume of the prostate. The proximal region is just this region here. And these regions are quite distinct at the level of marker expression as well as. Histology, so here if we go along the proximal distal axis, we have the paraurethral region has specific markers.
Here is the proximal region. It has a distinct Histology and is marked by specific gene expression of specific genes such as this one. PPP, one R1B. Then we have in the distal region other markers that are specific for distal luminal cells. The lume population here, but you will note there are also proxamol cells that are scattered about. They’re not very common, but you can definitely find them in the distal region and then in between. There appears to be a boundary where these regions meet.
If you perform electron micrography of sort of the boundary region, you can see that. These lumit lumayan lumpi cells actually appear to be distinct cell types, so the lume cells again are tall columnar secretory cells. The loopy cells, on the other hand, have a more cuboidal appearance, and they don’t seem to be particularly secretory. At the transcriptomic level, you can analyze the sort of relationships of these populations with each other.
So Luis Aparicio, postdoc INR Lab, who's done these computational analysis, has used. An approach based upon optimal transport theory to calculate Wasserstein distances between these populations, and then relate these populations to each other. You can see that Lumpi is sort of, you know, sort of at the center related to the distal lumenal populations, and PR you and basil. So in order to investigate the function of these populations, we've observed that there is greater...
NOTE Confidence: 0.8025036
00:22:10.018 --> 00:22:12.560 projector potential in the loom PPR.
NOTE Confidence: 0.8025036
00:22:12.560 --> 00:22:14.735 You and basil populations versus
NOTE Confidence: 0.8025036
NOTE Confidence: 0.8025036
NOTE Confidence: 0.8025036
00:22:19.100 --> 00:22:22.285 You can see that the distal luminal
NOTE Confidence: 0.8025036
00:22:22.285 --> 00:22:25.021 populations all have a low efficiency
NOTE Confidence: 0.8025036
00:22:25.021 --> 00:22:28.031 of formation of organoids lumpi as a
NOTE Confidence: 0.8025036
00:22:28.118 --> 00:22:30.960 much greater efficiency and PR you in
NOTE Confidence: 0.8025036
00:22:30.960 --> 00:22:34.670 Basel have a greater efficiency yet.
NOTE Confidence: 0.8025036
00:22:34.670 --> 00:22:36.935 We’ve also isolated these populations
NOTE Confidence: 0.8025036
00:22:36.935 --> 00:22:39.360 by flow cytometry and performed renal
NOTE Confidence: 0.8025036
00:22:39.763 --> 00:22:42.208 grafting assays so in combination
NOTE Confidence: 0.8025036
00:22:42.208 --> 00:22:43.675 with urogenital mesenchyme,
NOTE Confidence: 0.8025036
00:22:43.680 --> 00:22:45.996 these cells were formed renal grafts
NOTE Confidence: 0.8025036
00:22:45.996 --> 00:22:49.360 all be it with different efficiencies,
NOTE Confidence: 0.8025036
and then we can analyze the sort of cell types present within these graphs. So using the markers that we've identified that is specific for each population. In brief, the loom a another distal luminal. Cells can make more of themselves, but not the loom PNP Ru populations, where is the loom PNP? Ru populations can make all of the other different populations, so this supports a sort of a projector. Increased progenitor potential for loom PNP RU populations. So finally one can ask what is the relationship between...
the mouse and human prostate?

After all, anatomically they are quite different and histologically as well, so we've.

Analyzed three independent benign prostatectomy specimens at the single cell level, and again, we can see that there is a single basil population, but they're different luminal populations that we can relate to. The luminal populations that we see in the mouse, so there isn't a snare population.
that seems more closely related to
the distal lumenal populations.

A ductal populations more proxamol,
as well as a PR you like population.

Again,
this time in across species way we can
we can definitely show this relationship.

So the acinar cells are actually interesting.

Lee most closely related to
the ductal cells to loom
lumenal L cells in the mouse,

the ductal cells to loom
P&PRU 2P RU in Bloom,
P, etc.
So this analysis highlights loom L as
a population of interest in the mouse.
It perhaps is most closely related to the bulk of the human prostate epithelium. Yet the lateral lobe is understudied in the mouse, and particularly analysis of cancer models.

So now I’d like to turn to cancer a little bit and think about plasticity in advanced prostate cancer. So in the current spectrum of prostate cancer where we have treatment of with potent antiandrogens that are very efficient at suppressing androgen receptor function. Now, castration resistant prostate cancer is...
displaying a range of different entities. As sort of a spectrum that can be distinguished perhaps by different differential lineages, plasticity. So there is prostate cancer that is remains a our pathway positive it still expresses androgen receptor despite the presence of anti androgens. And then at the other extreme we have neuroendocrine prostate which is a are negative and. He’s most extreme forms can display a small cell phenotype that’s very aggressive and lethal, so there’s been considerable interest in the mechanisms of neuroendocrine
00:26:08.271 --> 00:26:10.325 differentiation in CR, PC, and so.
00:26:10.325 --> 00:26:13.469 There have been studies of CR PC that are
00:26:13.469 --> 00:26:16.277 trying to distinguish the different entities,
00:26:16.280 --> 00:26:18.572 and, for example, there is something
00:26:18.572 --> 00:26:19.718 considered double negative.
00:26:19.720 --> 00:26:22.012 That is a are negative and
00:26:22.012 --> 00:26:22.776 neuroendocrine negative,
00:26:22.780 --> 00:26:25.503 which is defined more by what it
00:26:25.503 --> 00:26:28.120 is not rather than what it is,
00:26:28.120 --> 00:26:30.200 but the relationships between these.
00:26:30.200 --> 00:26:32.624 Distinct entities is unclear,
00:26:32.624 --> 00:26:39.128 and it may be simple, maybe more complex.
00:26:39.130 --> 00:26:41.152 But there is widespread agreement that
00:26:41.152 --> 00:26:43.904 there must be a role for epigenetic
00:26:43.904 --> 00:26:46.700 reprogramming in this process, and so.
A range of studies have provided evidence that there's increase in ezh two as PRC.

Two activity in this spectrum of plasticity and recently flybot.

John Cody's lab has shown that PRC one activity is elevated in double negative prostate cancer etc.

But this is all involved studies either in cell lines or in human prostate cancer specimens, so it's difficult to sort.

You know at a more detailed level and in terms of mechanism as well.

We started with a mouse model that we...
had been analyzing in collaboration with Korea Body Schenz lab. So the NPP 53 mouse model uses inducible deletion of P-10 and P53. Of these animals develop a castration resistant prostate cancer that will display features of neuroendocrine differentiation and we were able to distinguish what we called focal neuroendocrine differentiation in which the neuroendocrine cells are not proliferative from overt nor endocrine differentiation which often displays a small cell phenotype. These are highly proliferative.
or endocrine cells.

However, since we use lineages marking here,

we could show that the neuroendocrine

cells are derived from a luminal cell,

so the initial.

Tumor induction was from luminal

cells and then we have.

Alright,

formation of CR PC neuroendocrine

differentiation and then there is

some type of proliferative switch

that we don’t understand that

results in this highly proliferative

neuroendocrine prostate cancer.

So because we use linear tracing here,

we provided evidence that in fact
this was a transdifferentiation of luminal cells to neuron can cells. So when we think about transdifferentiation, there’s sort of a fundamental question both in developmental as well as cancer context, which is what is really going on in terms of the pathways that result in transdifferentiation. Well, it’s possible that this change happens normally during development. Alternatively, it’s possible that this change
in identity occurs through a transition that is wholly or at least partially novel that doesn’t really occur in normal development, so there could be a hijacking of an alternative pathway or some other pathway or process that doesn’t occur in normal context. To understand this, of course it is first of all important to discover how neuroendocrine cells differentiate normally. It’s remarkable that there’s very little in the published literature about.
features of neuroendocrine cells in the normal prostate, enlarged likely because they are quite rare and what is also interesting is that there are different models have been put forth for their origin. So one model says that they actually are of epithelial origin and arise from Basel progenitors. Another model is that they arise from neural Crest and so Cedric Pompons lab published. That they came from basil cells and a more recent paper. From that they’re from their old Crest in both these papers use linear tracing.
In fact, we believe that both of these papers are incorrect and most likely they arise from an early urogenital epithelial progenitor.

So neuroendocrine cells are very rare. But what is make some particularly in the mouse, prostate is they’re highly asymmetrically distributed, so nearly all the owner can cells are proxamol.

They’re very rare distally. And remarkably, neuroendocrine cells, despite their rarity, are heterogeneous.
So about 80% of neuron can cells have adluminal like phenotype. They actually express androgen receptor remarkably and they express luminal cytokeratins and then another 20% of your endocrine cells are basil like they expressed basal cytokeratins and P. They can be detected very early in organic Genesis at burn. Many, most perhaps all Durand Prince cells are actually formed at prepubertal stages, and since neuroendocrine cells I didn’t mention this on previous slide are. But never divide there.
They appear to be post mitotic. We believe that they are made and are not subsequently generated. By lineages tracing, we believe that they have an epithelial origin, so using an NCX Cree driver we can mark most of the prostate epithelial cells and in fact the vast majority of neuroendocrine cells are marked in this experiment. So there’s more than 95% of the prostate epithelial origin. Finally neuroendocrine cells you know? Likely arise,
as has been shown in the lung through a pro neural pathway, in which sort of the master regulator at the top of of this of the sort of transcription factor hierarchy is ASE L1. So if we delete ACL one in the mouse prostate, we can actually recover mice that have prostates that completely lack your endocrine cells. And yet the prostate appears to be normal and there is a normal proximal region. So we do have a rare escaper cells in the Periorbital region which are likely due to incomplete deletion by index 3.1.
So our current model for the origin of your endocrine cells is that they likely arise from your original epithelial progenitor, although we haven’t excluded the possibility they arise from an early lumenal progenitor. But in either case progenitor activity coincides with the developmental stages in which androgen levels are very low, and we’re currently studying. The molecular properties of normal neuroendocrine cells. To understand you know in more detail their regulation.

Moving on to cancer,
we've used the NP 53 mouse model to generate organoid lines that displayed neuroendocrine phenotypes so. This is work from a talented postdoc in my lab, Jolly. She has used NP 53 tumors and established a large number of organoid lines, some of which have neuroendocrine features. As you can see here, this is the sort of primary tumor, this is heterogeneous. These are the organoids are established there, green because of the linear smirking. Here's a different line that you can here.
see the region of small cell Histology. And these organoids are heterogeneous. 
You can see that they have a neuroendocrine region as well as a non-mesenchymal region that is mesenchymal in nature. So this can be more clearly revealed by marker analysis. 
So here’s a line that’s relatively homogeneous. It expresses Synaptophysin and Chromogranin so it has a neuroendocrine phenotype. Here’s a different line that is more heterogeneous there that has sort of mixed expression of neuroendocrine markers as well as androgen receptors.
To some extent, it actually has a double positive or African phenotype. This is a heterogeneous line that I showed you earlier, so it is intermixing of neuroendocrine cells and non-endocrine cells that express androgen receptor. Here is a different heterogeneous line again with a similar intermingling. So these new rendering lines, whether homogeneous or heterogeneous or highly stable during passaging, they can be passaged for.
more than 20 passages, and the heterogeneous lines will maintain their heterogeneity. So we can analyze the heterogeneity using single cell RNA sequencing and so this is the first line I showed you, Interestingly the clusters are sort of grouped together, and we see a similar arrangement of clusters. And this is this is the other heterogeneous line, and we see a similar arrangement of clusters. So the heterogeneity of these organize is striking because it suggests that we’ve been able to capture
much of the spectrum of CR PC within organoids established organoid lines. So what can we do with these organoid lines? Well, we can do a number of things. One thing is we can examine, you know, sort of the epigenetic marks that are displayed in these organoid lines. For example, we’ve been pursuing cut and tag analysis here, looking at H3K27 trimethyl. So this is the mark deposited by PRC 2. And so I’ll just show you just little this little tidbit here.
actually the non neuroendocrine lines appear to have a somewhat higher level of H3K27 trimethyl than the neuroendocrine lines. So that’s something interesting that we are currently following up on. We’ve also been collaborating with Andrea Califano’s laboratory, which has developed a set of computational systems approaches to identify master regulators that drive biological processes. One of the analytical approaches that they’ve developed is known as Meta Viper, where we can take single cell
NOTE Confidence: 0.84401006
00:37:52.376 --> 00:37:54.519 RNA sequencing data and analyze
NOTE Confidence: 0.84401006
00:37:54.519 --> 00:37:56.704 this to infer protein activity
NOTE Confidence: 0.84401006
00:37:56.704 --> 00:37:58.990 at the single cell level.
NOTE Confidence: 0.84401006
00:37:58.990 --> 00:38:02.147 So Alessandro Vasi Evo in Andre’s lab
NOTE Confidence: 0.84401006
00:38:02.147 --> 00:38:05.028 postdoc in Andre’s lab has done this,
NOTE Confidence: 0.84401006
00:38:05.030 --> 00:38:07.352 and again using the same organoid
NOTE Confidence: 0.84401006
00:38:07.352 --> 00:38:09.958 line as I showed you earlier
NOTE Confidence: 0.84401006
00:38:09.958 --> 00:38:11.918 here by protein inference,
NOTE Confidence: 0.84401006
00:38:11.920 --> 00:38:14.937 we can see again clustering of ARPU.
NOTE Confidence: 0.84401006
00:38:14.940 --> 00:38:17.860 See any PC and NPC.
NOTE Confidence: 0.84401006
00:38:17.860 --> 00:38:24.400 The the RPC cluster is elevated.
NOTE Confidence: 0.84401006
00:38:24.400 --> 00:38:27.190 Using an androgen receptor signal is
NOTE Confidence: 0.84401006
00:38:27.190 --> 00:38:29.547 is enriched for androgen receptor
NOTE Confidence: 0.84401006
00:38:29.547 --> 00:38:32.061 signature the any PC cluster is
NOTE Confidence: 0.84401006
00:38:32.061 --> 00:38:34.497 enriched for a neuroendocrine signature
NOTE Confidence: 0.84401006
and when we can predict master regulators using this sort of approach.

Notably one of the newer endocrine master regulators that’s predicted is ACL one.

This is a method that we’re employing to identify candidate intrinsic drivers of neuroendocrine differentiation that we’re currently seeking to validate in functional assets.

One of the sort of interesting questions we can ask is does trans differentiation occur at some level in organoid cultures and we have some evidence that that might
one way that we’ve been looking at this is using single cell a tax seek to examine chromatin Accessibility, and we can see that this is the same line again by single cell, a taxi there, seven clusters, these clusters are. Have open chromatin at chromogranin. A neuron can marker these in a R and what you can see is that there is one cluster here that has open chromatin for both. Looking at the genomic locus so this cluster seven has accessible chromatin.
at both chromogranin A&AR and so.

This cluster, we believe,

corresponds to a potential transitional

population in the process of

neuroendocrine differentiation.

We’ve also been trying
to assay this directly,

so this is very preliminary data,

but we can isolate non your
endocrine cells by flow cytometry.

Mark them with expression of RFP and
then culture than honor in cells,

and neurons can sell separately for
several passages and they maintain

their non **** couldn’t border and can

phenotypes if we coculture them together.
However, we now see their rare cells that our RFP positive. But now express neuron could markers such as synaptophysin or Chromogranin A and interesting Lee. They maintain the expression of the Menton. So these appear to be a transitional cell, since phenotype seemingly corresponds to the what the a taxi might predict. OK, so these are some of the approaches that we’ve been employing to study language plasticity in prostate cancer. In the remaining 10 minutes or so,
I'd like to switch over to bladder cancer and explain how we've been using organize to study plasticity in bladder cancer. So bladder cancer is of course a major health problem. It's quite understudied. The normal bladder contains again sort of three epithelial cell types, as it were basil cells, intermediate cells, and umbrella cells, and bladder cancer can be roughly divided into non muscle invasive disease and muscle invasive disease. Historically,
these have been considered to be almost distinct entities, and it’s unclear what the relationship actually is so. There are two forms of non muscle invasive bladder cancer, papillary and carcinoma insight, two and carcinoma insight two has been considered to be sort of the precursor to muscle invasive disease. However, there is also some evidence that papillary disease can progress to muscle invasive disease, so we’ve been interested in studying
progression of bladder cancer and.

To pursue this, we've actually established patient derived bladder tumor organoids, and these have been established through collaboration with urologists who perform transurethral resection’s. So what they do is they sort of go in and extract, sort of like the tops of these tumors here. This is what they actually view through the cystoscope. This might look a little uncomfortable for men in the audience, but.
take these samples and we can establish organoid lines in culture, which we can seriously passage. These organoids can also be grafted. Orthotopic Lee. This orthotopic grafting it uses ultrasound guided implantation into the bladder wall which is a very efficient process so we can readily interconvert organoids into Xena graphs. We can also take the Xeno grafts and convert them back to organoids, so all of these. Together with the parental tumor can be analyzed by sequencing or histopathology etc. So this just shows you some of the organoid
lines that we established and what is, I think, evident is that the organoids and the Xeno grafts retained the characteristic histology of the parental tumor. We also can establish organoid lines that have less common histological variants, such as squamous cell carcinoma. In recent unpublished work, we’ve now increased our bio bank to approximately 50 organoid lines. We’ve been able to establish organized lines from cystectomy samples, as well as from transferring for receptions, and have also a stab Liszt several lines that contain variant histologies.
So in collaboration with David Solids Group at Memorial Sloan, Kettering Owen, the previous pathology was in collaborate with collaboration with him at all Media Memorial. We’ve analyzed these organoid lines molecularly using the targeted sequencing platform at Memorial MSK impact. We sequenced the organoids parental tumor and normal bloods and we can show generally that the organoids. Display mutational profiles that are concordant with that of the parental tumor. We can examine.
Sort of the mutational profiles of these organoid lines, which really recapitulates sort of the distribution of mutations in human bladder cancer. So we can see that among the common mutations we see mutations in a lot of epigenetic regulators which are frequently mutated in bladder cancer, such as KDM 6A KMT 2C and 2D. As well as error 1A. Interestingly, we also see were also able to capture mutations that are relatively rare but interesting in bladder cancer, such as mutations and ERB B2 and of note we have very few nations in RB,
so many bladder cancer cell lines were established from metastatic bladder cancer and contain RB mutations, whereas are organoids. Are generally established from non muscle invasive bladder cancer or earlier stages of muscle. Invasive disease and lack RB mutations. So what can we do with these organoid lines? One thing we can do is we can examine their drug response, and of particular interest were able to establish organoid lines from patients in a longitudinal fashion. So patients will often undergo
transurethral resection, be treated,

and then sometime thereafter,

their tumors will unfortunately recur.

And then we have an opportunity to

establish another organoid line.

So here’s an example of a patient.

And a pair of organoid lines where

patient was not otherwise treated.

So the tumor was removed,

but the patient was not otherwise treated,

and we can see in terms of

response to a range of different

drugs that Interestingly,

the organoid lines display nearly

overlapping drug response profiles.

However,
In this case, the different patient was treated with Mitomycin C and BCG. The tumor relapsed after over a year, and now the recurrent organoid line is much more resistant to a range of different drugs. However, displays similar responses to other drugs, so. It’s of interest to us to understand how. Drug response has altered the properties of these organoids. So one thing we’ve started to do is to perform single cell analysis here of this recurrent pair, and Interestingly,
the recurrent organoid line is actually much more heterogeneous than the sort of the parental of the order online from the parental tumor. And what is interesting is if you re aggregate these together now you can identify a cluster that is actually in common between both the between the 1st and the 2nd organoid lines so. This cluster, we believe, corresponds to a transitional population and we can identify markers that are specific for this transitional population, so our hope here is that we can use
utilized this pair of organoid lines and other examples of recurrent disease that we have to sort of replay in organoid culture. The events that take place during the emergence of treatment. Resistance.

So finally I'd like to address the issue of tumor progression in organoid, so non muscle invasive disease you know can be classified into two lumenal categories as well as a basil category. Muscle invasive disease is more complex and again as I mentioned earlier, the relationship between these.
entities has been somewhat unclear. However, there is a question of whether you know if we have progression, whether there might be a switch and subtype specifically. From sort of Class 2 luminal tumors to Basel to a basal squamous muscle invasive tumor. So we believe that we can recapitulate this in organoid culture. So many of our organoid lines are phenotypic least able and culture. If you look at different markers there stable and organoids as organoid Xena, stable and organoids however. Sort of a little over a
00:49:28.500 --> 00:49:30.490 majority of the organoids from.
00:49:30.490 --> 00:49:32.590 Non muscle invasive tumors displayed
00:49:32.590 --> 00:49:34.690 this sort of phenotypic plasticity.
00:49:34.690 --> 00:49:37.690 This is a they start with the luminal
00:49:37.690 --> 00:49:39.934 phenotype here and they become
00:49:39.934 --> 00:49:41.830 basil during organoid culture.
00:49:41.830 --> 00:49:44.350 But notably this phenotype can be
00:49:44.350 --> 00:49:46.030 largely reversed by xenografting,
00:49:46.030 --> 00:49:48.368 so we believe this there’s an effect
00:49:48.368 --> 00:49:50.441 of the tumor micro environment
00:49:50.441 --> 00:49:54.425 luminal differentiation.
00:49:54.430 --> 00:49:57.542 In fact can reverse it but if we
00:49:57.542 --> 00:50:00.409 remove the tumors again from the.
00:50:00.410 --> 00:50:03.406 In a graph and culture miss organoids,
they will again undergo the space little differentiation.

So John Kristen Terrific Post Document Lab has pursued a small molecule screen to examine organoid lines that display plasticity to see if it’s possible to revert it, and he identified one big hit here GSK. Else one, which is a KTM one inhibitor and it is able to partially revert this plasticity, so the.

I’m not showing this here, but if he knocks out KTM one day, he can also see this effect. So.

He is currently investigating
the mechanisms by which KTM 1A regulates this transition.

In parallel, he’s also been pursuing a taxi. Can Alesis to examine sort of the epigenetic States and eventually the epigenetic marks that are associated with this plasticity.

So if you examine, for example, these three organoid lines, this is adluminal line. This is a basil line and this is what we call a plastic line.

The plastic line looks like a basil line, although it started with a luminal phenotype.

So we’ve performed a taxi.
Can Alesis and we can cluster these so you see that the lumenal lines clustered together. The basil lines clustered together and these are the plastic lines which in the first principle component cluster together with basil. But they’re separated along the second principle component. So now if we look at, you know, sort of genomic tracks, you can see that at basil markers such as keratin 14, the basil lines of course have open chromatin and the plastic lines also have open chromatin, which of course is to be expected.
because they have a basal phenotype.

But what’s interesting is that lumenal markers, such as 
gotta three. We see that the plastic organoid lines 
seem to have partially open chromatin, so they retain an epigenetic 
memory of their lumenal origin. So we are currently pursuing 
studies to determine whether we can 
actually detect this epigenetic 
memory in human prostate tumors, 
which might indicate. A specific pacifically base of the 
basal squamous category to determine 
NOTE Confidence: 0.8060535
whether they may have in fact originated from more luminal tumor. Finally, we’re also pursuing motif discovery approaches to identify candidate transcription factors that might be driving this transition, and we’re coupling this with other computational approaches. We think that this really is modeling something that’s happening during, you know, sort of transition to muscle invasive disease because. As has been noted in breast cancer for Andy Walt, what we’ve found is at the at the invasive front of these tumors.
Now we see the expression of a basal marker cytokeratin 14 at the invasive front, but this is not expressed within the tumor body.

So in closing, then I’d like to underscore that we think these organoids represent a model system for studying tumor plasticity. We can identify transitional populations in patients in order rates from patients with recurrent disease, and we think that the sort of plasticity we’re studying culture can reflect processings of disease progression in vivo, and we’re using computational
A systems approach is to identify the drivers of plasticity. And more generally, we believe that organoid models are incredibly useful because they will allow mechanistic studies of complex questions in cancer biology that may be inaccessible. Of course, the work that I've described involved large team of terrific scientists really did all the work. So notably, Laura Crowley is a graduate student who led the single cell.
analysis of the prostate epithelium together with Francesco Comma, Boolean, former postdoc Moshe Botton, now at George Washington University. The work on normal neuroendocrine differentiation was performed by graduate student gave alone. And the prostate organized by Jolly a terrific postdoc in the lab. Then former lab members soup soup Lee started the bladder organized project and I mentioned John Kristen is a current post Doc who’s played a major role in continuing this project. We’ve had terrific collaborators.
00:55:26.930 --> 00:55:29.170 Roll rabadan Andrea Califano for
NOTE Confidence: 0.83516306
00:55:29.170 --> 00:55:30.514 computational systems biology.
NOTE Confidence: 0.83516306
00:55:30.520 --> 00:55:31.418 Jim Mckiernan,
NOTE Confidence: 0.83516306
00:55:31.418 --> 00:55:33.663 whole team of talented urologists
NOTE Confidence: 0.83516306
00:55:33.663 --> 00:55:35.010 who provided samples.
NOTE Confidence: 0.83516306
00:55:35.010 --> 00:55:37.698 We’ve collaborated with Korea Body Shen,
NOTE Confidence: 0.83516306
00:55:37.700 --> 00:55:39.950 in analysis of Mouse models,
NOTE Confidence: 0.83516306
00:55:39.950 --> 00:55:42.260 which how Lewin, epigenetic analysis.
NOTE Confidence: 0.83516306
00:55:42.260 --> 00:55:45.374 Need help search for pathology as
NOTE Confidence: 0.83516306
00:55:45.374 --> 00:55:49.298 as well as Max load at Cornell.
NOTE Confidence: 0.83516306
00:55:49.300 --> 00:55:51.052 For pathological analysis and
NOTE Confidence: 0.83516306
00:55:51.052 --> 00:55:53.242 a great group of collaborators
NOTE Confidence: 0.83516306
00:55:53.242 --> 00:55:55.498 at Memorial Sloan Kettering,
NOTE Confidence: 0.83516306
00:55:55.500 --> 00:55:58.250 David Solid Hickman to Marty
NOTE Confidence: 0.83516306
00:55:58.250 --> 00:55:59.900 and Barry Taylor.
NOTE Confidence: 0.83516306
00:55:59.900 --> 00:56:01.846 So thank you very much and I’ll
00:56:01.846 --> 00:56:03.980 be happy to take any questions.

00:56:05.490 --> 00:56:07.270 Thank you very much, Michael.

00:56:07.270 --> 00:56:09.734 That was a wonderful talk and really

00:56:09.734 --> 00:56:12.273 amazing to see all of these models

00:56:12.273 --> 00:56:14.385 and everything that can be done

00:56:14.390 --> 00:56:16.810 with all of these different models.

00:56:20.040 --> 00:56:22.146 I would like to remind the audience

00:56:22.146 --> 00:56:24.720 that they can type questions in

00:56:24.720 --> 00:56:26.904 out and ask them to Michael.

00:56:26.910 --> 00:56:29.718 I will get started

00:56:29.718 --> 00:56:31.590 with a question Michael.

00:56:31.590 --> 00:56:34.950 One of the things that you see in

00:56:34.950 --> 00:56:38.472 the organoids and I was thinking in

00:56:38.472 --> 00:56:41.062 particular about the prostate cancer
ones is there's a the heterogeneity and I was just wondering whether you see shifts in the heterogeneity or of the organoids themselves when you use different types of media. So can you see shifts based on how you grow them? And then I was also wondering whether you have applied certain types of therapies to those prostate cancer organoids and whether you see changes in that heterogeneity and shifts when you use different different types of treatments. OK, so Katie, that's a great question.
00:57:24.462 --> 00:57:26.190 question regarding media so.
00:57:26.190 --> 00:57:29.060 One of the things that I forgot
00:57:29.060 --> 00:57:32.527 to mention is that we use our own
00:57:32.527 --> 00:57:35.387 sort of homegrown media for all
00:57:35.387 --> 00:57:37.867 of these organoid experiments.
00:57:37.870 --> 00:57:41.662 This is a complex medium containing
00:57:41.662 --> 00:57:44.190 hepatocyte media and serum.
00:57:44.190 --> 00:57:46.578 You know we.
00:57:46.580 --> 00:57:50.132 Develop this years ago to grow
00:57:50.132 --> 00:57:53.169 mouse prostate organoids so it’s
00:57:53.169 --> 00:57:55.974 quite different from the ENR
00:57:55.974 --> 00:57:58.960 based media that many groups
00:57:58.960 --> 00:58:02.110 use to pursue organoid assays.
00:58:02.110 --> 00:58:05.170 There are although.
00:58:05.170 --> 00:58:07.900 You might imagine that some of the
growth factors involved are in common.

There are undoubtedly differences between the media compositions in terms of what’s actually going on.

We know that, anecdotally, for bladder tumor organoids that it is probably easier to establish patient derived organoids using our media than in our based media.

And we also know that you can transition organoid lines from one media to the other, but it may not be that straightforward.

So we have some experience with DNR based media, but all the analysis that I’ve showed you today were done in our sort of homegrown media.
So do we observe shifts in composition in different media?

We have not really examined that. In part because it can be different, it can be difficult to transition organ lines from one medium to another. In terms of drug treatment, we have only started to do this with respect to the prostate organoids the. Bladder organizer, something that we’ve been examining in more detail. We’ve been particularly interested in mechanisms of cisplatin resistance, which, of course is of considerable
translational interest.

So those are studies that are currently being pursued to examine how cisplatin treatment alters.

Or the phenotype, and perhaps the heterogeneity of the organized, but I don’t have any results yet.

Great, thank you.

There’s a question from Gefsky.

Are you been able to analyze human lumenal bladder tumors for plasticity, markers and correlate those results with subsequent development
So that’s a great question. Obviously we want to extend what we’ve been doing in organoid culture to human specimens, so.

I have to confess that this work that we’ve only recently gotten started, so we don’t.

Part of the problem is actually having a cohort of patients that’s suitable for this.

Yeah, we are now in the process of trying to assemble patient cohorts where we can actually.

Have samples are sort of launch eternal from patients who have progressed,
say from high grade non muscle invasive disease to muscle invasive disease. Assembling these cohorts is very nontrivial. Fortunately we are part of a large collaboration. Led by Corey Body Shan, together with collaborators at Memorial Sloan Kettering and at Johns Hopkins, so collaborates at more Memorial and others to try.
to gather together the. Cohorts that are essential to address this type of question because they don’t currently exist, and these types of samples are rare.

Thank you we have another question from Mike Hurwitz who says great talk within the different luminal subtypes. Do you think some are more likely to develop into cancer? Any correlate in human prostates? OK, so this is a question about prostate and of course we’re very interested in cell of origin. You know, we’ve always been sort of
dissatisfied with our previous analysis of Cell of Origin because you know you have luminal cells and basil cells. There’s only so much you can say, but we think that there’s still something to explore here because. This is well known phenomenon in which you know 85 to 90% of prostate cancer patients. With sort of intermediate risk disease, you know a will actually have indolent. Prostate cancer, whereas the remaining 10 to 15% of patients actually have aggressive disease and it’s difficult to distinguish between the indolent and aggressive tumors and
Despite a lot of molecular analysis, they haven’t tremendously improved over. Just simple histological police and grading. So we think that it remains possible that cell of origin could explain at least partially. The difference between indolent and aggressive disease. And so that’s something we’re very interested in pursuing. The. We know already that you know from the literature that both proximal and distal luminal cells can be cells of origin in mouse models. But that does not necessarily answer
the question because you know they may be different in terms of their phenotype or response to treatment or. Ultimate outcomes so you know we’re in the process of pursuing these types of studies. It’s not really clear what’s going on in the human prostate. And again, I think we’re just scratching the surface in terms of understanding the relationship between the lumenal populations in the mouse and the populations of the human. There’s a lot more work that needs to be done there. Well, thank you very much Michael for
01:04:33.599 --> 01:04:36.039 this visit for this fascinating talk.
01:04:36.040 --> 01:04:38.830 I know it made me think of a lot of things or some parallels in the world of lung cancer.
01:04:41.371 --> 01:04:44.410 So it was really great to think about this so thank you very much for visiting us today and thank you everybody also who joined and have a wonderful afternoon.
01:04:46.755 --> 01:04:49.204 about this so thank you very much for visiting us today and
01:04:49.204 --> 01:04:51.256 much for visiting us today and
01:04:51.333 --> 01:04:53.727 thank you everybody also who joined and have a wonderful afternoon.
01:04:55.700 --> 01:04:56.440 Well, thank you Katie. Thank you every.