Of hosting today and we are incredibly thrilled and delighted that we were able to induce Doctor Gigi Lissonota to be our speaker today.

Doctor Lozano graduated from Pan American University in Texas, did some work at Oakridge Laboratories, got her PhD at Rutgers, and then went to Princeton, where she was a postdoc with Arnie Levine. Immediately following that, she was recruited to MD Anderson, and in her long tenure there she has done. Path breaking work on P53.
She's recognized for enormous contributions that include the recognition that P53 works as a transcriptional activator. Many, many findings regarding the ways that MDM two and MDM three regulate P53. Extensive use of transgenic models to understand these mechanisms. Better definition of specific P53 loss and gain of function mutations and their effects on P53's biology as well as studies of the P53 transcriptional program. She's a member of the National Academy of Sciences and the National Academy of Medicine and at MD Anderson.
She is now chair of the Department of Genetics and Hubert L. Olive Stringer, distinguished chair in oncology. In the division of Basic Science Research at MD Anderson, so I don’t want to talk for a long time because I’m really eager to hear what you have to say. So thank you very much for joining us and I will just say to the audience. You can use the chat function to enter your questions or we can. I believe we can unmute people at the end if we need to. So with that do welcome.
Thank you, thank you very much, Barbara. For that introduction I hopefully now I’m sharing my screen. Can you see it? Weekend OK super Alright so anyway thank you Barbara. It’s it’s fun to always visit a place and now during covid we’re doing these virtual seminars but hopefully at the end I’ll be able to address some of the questions that people might have. What I thought I would do today is described some of the models that I think have to find some of the basic real basic understanding of
00:02:37.020 --> 00:02:39.290 the P53 tumor suppressor pathway.
NOTE Confidence: 0.83147156
00:02:39.290 --> 00:02:41.796 So I’ll get started with my disclosures.
NOTE Confidence: 0.83147156
00:02:41.800 --> 00:02:44.626 I am on the Scientific Advisory
NOTE Confidence: 0.83147156
00:02:44.626 --> 00:02:46.510 Board for PMV Pharma.
NOTE Confidence: 0.83147156
00:02:46.510 --> 00:02:48.580 So the P53 pathway.
NOTE Confidence: 0.83147156
00:02:48.580 --> 00:02:51.356 This is my myopic view of the pathway
NOTE Confidence: 0.83147156
00:02:51.356 --> 00:02:53.550 and it really highlights.
NOTE Confidence: 0.83147156
00:02:53.550 --> 00:02:56.034 I think some of the critical
NOTE Confidence: 0.83147156
00:02:56.034 --> 00:02:57.690 features of the pathway.
NOTE Confidence: 0.83147156
00:02:57.690 --> 00:03:00.511 First and foremost people decree is present
NOTE Confidence: 0.83147156
00:03:00.511 --> 00:03:03.478 in very low levels in normal cells,
NOTE Confidence: 0.83147156
00:03:03.480 --> 00:03:05.550 but any kind of abnormality
NOTE Confidence: 0.83147156
00:03:05.550 --> 00:03:07.206 that the South senses,
NOTE Confidence: 0.83147156
00:03:07.210 --> 00:03:08.674 hypoxia, DNA damage,
NOTE Confidence: 0.83147156
00:03:08.674 --> 00:03:10.626 inappropriate activation of an
NOTE Confidence: 0.83147156
00:03:10.626 --> 00:03:12.485 oncogene will stabilize that
P53 protein and then P53 in turn functions as a transcription factor. To activate hundreds of genes and I’ll show you some of those experiments in a little bit, but some of the genes that P. 53 is known to activate is P21 for example, which is a inhibitor of the South cycle. It also induces senescence program and P53 can activate a slew of gene sick, initiate a pop ptosis and it also activates. Genes are involved in changing the metabolic functions of a cell. Now when P 53 is activated, if the cell is allowed to survive
and proceed,
NOTE Confidence: 0.83147156
P53 has to activate this protein
NOTE Confidence: 0.83147156
called MDM 2,
NOTE Confidence: 0.83147156
which is an E3 ubiquitin ligase
NOTE Confidence: 0.83147156
that targets P53 for degradation
NOTE Confidence: 0.83147156
and basically removes the Peachtree
NOTE Confidence: 0.83147156
levels back down to normal.
NOTE Confidence: 0.83147156
So if you just.
NOTE Confidence: 0.83147156
Think about what people think we can do.
NOTE Confidence: 0.83147156
We can do so much in getting arrested,
NOTE Confidence: 0.83147156
phallic it, killed itself,
NOTE Confidence: 0.83147156
and it can also induce its own
NOTE Confidence: 0.83147156
inhibitor to allow the cell to survive.
NOTE Confidence: 0.83147156
And even though there’s so much
NOTE Confidence: 0.83147156
work in the pizza tree killed,
NOTE Confidence: 0.83147156
we still don’t understand all of the
00:04:26.952 --> 00:04:29.238 cues that determine which of these pathways Peabody Creek and activate.

00:04:29.238 --> 00:04:31.208 But because it has all these functions, it is a critical tumor suppressor, and it is the most mutated gene human cancers.

00:04:33.536 --> 00:04:35.528 So what I showed here is what the field called the Manhattan Plot, and it was developed by Magali Olivia.

00:04:35.530 --> 00:04:39.410 So across this axis are 125 genes that are commonly mutated and cancers.

00:04:37.858 --> 00:04:39.410 And then across this axis here are 36 different types of cancers, and there’s some features that stand out.

00:04:42.274 --> 00:04:44.559 So across this axis are 125 genes that are commonly mutated and cancers.

00:04:42.274 --> 00:04:44.559 And then across this axis here are 36 different types of cancers, and there’s some features that stand out.

00:04:44.560 --> 00:04:47.325 and it was developed by Magali Olivia.

00:04:47.330 --> 00:04:49.906 So across this axis are 125 genes that are commonly mutated and cancers.

00:04:49.906 --> 00:04:52.258 and there’s some features that stand out.

00:04:52.258 --> 00:04:54.988 But this is the one I want to
These are mutations in the P53 tumor suppressor. So almost all cancers mutate. But P53 pathway is inactivated by multiple mechanisms and I show here in some of the different cancers and how they inactivate piece of debris. So high grade, serious ovarian carcinomas, mutations in P53 are the most common. In liposarcomas it is upregulation of MDM2, if it could be like a San. About 100% of these liposarcomas inactivate the pathway by overexpressing MD.
In the new Asia glioblastoma, big Sweet come interested in glioblastomas. Recently, the P 53 gene is altered, deleted in approximately 1/3 of glioblastomas in the MDM to an Indian. For genes are upregulated in about it order and this is a mutually exclusive relationship. So if P53 is mutant, MDM 2 doesn’t have to be upregulated. And the other thing I want to point out in glioblastomas is that we have about half of these tumors that have neither mutations and piece upregulation of the MDM.
Two and MDM four inhibitors of P53.

And so since I really believe that the P53 pathway has to be undermined in the development of all cancers, I think that there’s a big hole here that we have to understand in more detail.

So today’s start is really going to concentrate on just a few proteins, and I’m not going to have time to show you a whole lot of data, but I thought I would use this slide to highlight some of the important functions that I will discuss with you today. So MDM two is an inhibitor of P53. It’s an E3 ubiquitin ligase and
00:07:01.786 --> 00:07:03.350 target speakers for degradation.

00:07:03.350 --> 00:07:07.130 MDM Four also inhibits P 53.

00:07:07.130 --> 00:07:09.888 It doesn’t have any E3 ligase function,

00:07:09.890 --> 00:07:12.110 but it actually facilitates and makes

00:07:12.110 --> 00:07:15.010 MDM two or better yet riveting ligase,

00:07:15.010 --> 00:07:17.100 although it also has independent

00:07:17.100 --> 00:07:19.735 functions of MDM two and can

00:07:19.735 --> 00:07:22.546 this relationship does MDM 2 New Four

00:07:24.549 --> 00:07:27.546 guy free transactivation domain.

00:07:27.546 --> 00:07:30.280 also conform hetero dimer and that

00:07:30.280 --> 00:07:33.028 header dimer is critical in embryo

00:07:33.028 --> 00:07:35.440 development to inhibit P 53 and then,

00:07:35.440 --> 00:07:36.700 as I indicated,

00:07:36.700 --> 00:07:39.220 and will discuss in some detail,
P53 can activate the Indian two promoter so it can up regulate MDM two in inhibited so levels.

Another important concept that I'll mention very briefly, maybe at the end is that MDM two can also inhibit. A mutant P53 protein and that's because these mutant proteins have mutations in the DNA binding domain but retain a transcriptional activation domain.

But the important point that I want to make here is even though this mutant make here is even though this mutant can be targeted by MD M2 and MD M4. It is mutant in so can no longer feed back it up.
Regulate MDM two so with time in our in vivo models we find that these people period this can become stabilized 'cause there's insufficient MDM two to down modulate the protein levels and in a few minutes you'll see how important it down modulation is. So the outline of my talk today is I'm going to talk about some of the models that showed us how exquisitely sensitive P 53 is to inhibition via video 2. To tell you what the molecular responses to people three activation in vivo and last but not least, I'm going to describe some of the new
cancer models that were working with
that expressed mutant P53 proteins.
So let’s first talk about the MDM proteins and their innovation P.
So along time ago and now we.
Attempted to make an Indian to know Mouse and it’s just not possible and the reason is not possible is because an MDM two null embryo just prior to implantation is APOP totic.
This embryo stained with the tunnel essay in every salad. This embryo is is dead.
And at the time we knew that MDM two interacted with P.
but we really didn’t know how
important interaction was and what
we did was test the importance of P53 in this little embryo by
crossing 2P53 miles and we completely rescue this phenotype.
These Meister born and are perfectly normal except now because they lack P 53 they have it.
So with with this experiment indicates is that what MDM two is doing in these embryos is upregulating P53,
which is preventing the normal development of these embryos.
MDM fours are related MDM, two protein that aren’t Johansson discovered.
And since MDM two has such a unique relationship with P53 we decided that we would make the MDM for knockout but weren’t sure what to expect. But in MD for knockout is also embryo lethal a few days after the Indian to know but again that phenotype is rescued by deletion of P53 and we’ve also made mice that have no MDM to know also made mice that have no MDM to know Indian for Nokia 53 and these mice. The viable they have Pizza 3 two or phenotypes because they lack P 53. So at least physiologically, in the mouse, the two most important functions of these empty in proteins is to keep
P 53 levels low during homeostasis.

OK, so we have these MDM two heterozygous in these MDM for headers I customized. They have only one allele of each of these two genes. In these mice are perfectly normal and running around, but as I indicated in my introductory slide. P 53 is a DNA damage response protein, and so we wondered if there was any phenotypes in these mice. If we irradiated them, if we damaged their DNA, and sure enough,
we saw a beautiful phenotype.
So the MDM two in the MDM,
two Ambien for headers agasse mice are sensitive to low dose ionizing radiation.
So in this experiment what we’ve done is irradiated mice with six Gray and the black line above.
Here is a normal mouse.
That for 50 days just ignores.
Six grade radiation, but the headers.
I guess.
Mice MDM four in Red and MDM two and blue are dead by three weeks of age.
Importantly,
if we now move P53 from this system,
we completely rescue these phenotypes.
So this is the rescue. The Indium MDM, two heterozygous mouse. And here’s the rescue of the Indian for headers ecospace. So even though. The MDM two and MDM for heterozygotes mice have sufficient levels of the Zambian proteins to maintain homeostasis with damage. There’s just not enough of these proteins to return degree back to normal levels. The next experiment that I want to tell you about is the importance of this feedback loop. So as I indicated, MDM two is regulated by P53.
There are two peaks decree binding sites in the P2 promoter.

There are people, three dependent, so people free byansi sequences and activates MDM 2.

And So what we decided to do is to ask how important was this feedback loop in regulating P53 levers? OK, so we made point mutations and I show so we made point mutations and I show here the different point mutations because we didn’t want to disrupt the architecture of the promoter.

We just wanted to disrupt MD PhD degree binding to the end game to promote it.
So these experiments in the bottom bar chip data that basically show that P53 cannot find this mutant promoter, which we call P2P2, and this is different promoter that shows our assays are working in P53 combined. The pull up remote. OK, so we made these point mutations so we made these point mutations and we were surprised that are most was perfectly normal. We really thought that this feedback loop is going to be critical for regulation of P53. The mice are fine, but again,
as in the previous case, their exquisitely sensitive to radiation, so this is the same experiment that I showed you before we rated it the we irradiated the mice with six grade. Normal mice. MDM two heterozygotes. Don’t care about this dose, but you can see that the P2P2 homozygous mice that no longer have this feedback loop are dead for the most part. These animals are actually dying because of the complete ablation of the ball mirror, so here’s a heterozygous irradiated mice
and you can see that at 12 days you have some disruption of bone marrow function, but it’s still viable, whereas in the mice that lack the feedback loop, there’s a complete ablation of Humana Pelisses, and this is again a P53 dependent. We completely rescued this phenotype so if we take out just one illegal appeal 53. We completely rescued this phenotype so we can rescue this phenotype with complete deletion of P53 or header zygosity 5053. So with these experiments are beginning to tell us is that there’s this.
There is this important relationship between MDM2 and P53, and then there’s an important balance that has to be maintained for survival after DNA damage. The last experiment that I’ll show you here is our attempts to try to understand which pathway downstream of P53 is important for this phenotype. So I’ve already told you that heterozygosity Peachtree rescues the phenotype. We also generated mice with deletion of P21, which is the cell cycle inhibitor and that had no effect on the phenotype. Those mice are also very sensitive to radiation,
and then we also deleted Puma, which is one of the APOP totic targets of P53.

And here you can see that there was a complete rescue of this hematopoietic defects. So in this scenario it appears that it is the APOP totic function opekta degree that is killing. These hematopoietic stem cells. So those are just a couple of the numerous experiments being done to evaluate the relationship between MDM24 and P53, and it’s just an exquisite relationship.
You need sufficient MDM 2 for survival.
And if you have too much and you too MDM for deletion of 53 deletion of downstream effectors of P53 can rescue those lethal phenotypes.
Now come. Because. MDM two is so lethal early.
During embryo Genesis, one of the experiments we wanted to do is really ask about an adult mouse and how important is MDM two in different tissues at different times, and we’ve used, we’ve generated this conditional allele of MDM two and this is using
The LOX P system so these two lacks besides encompass two of the accents that code for the major finding. Region 2P53 so this conditional Leo then allows us to delete MDM two in any tissue that we want to. And we’ve generated number of tissues and experiments that way. But what I want to show you is what happens when you globally remove MDM two in the whole months. So as the title says, Indian too lost in the adult is results in a lethal phenotype. So what we’ve done here is we’ve
00:18:02.985 --> 00:18:05.212 used a Cree transgene that is tamoxifen inducible so this is a mouse that has one of the conditional alleles and it has the other allele missing, so it’s single recombination event is going to create an M2 normal or not sell in.

00:18:07.217 --> 00:18:10.296 this is a mouse that has one of the conditional alleles and it has the other allele missing, so it’s single recombination event is going to create an M2 normal or not sell in.

00:18:12.126 --> 00:18:13.956 so it’s single recombination event is going to create an M2 normal or not sell in.

00:18:16.324 --> 00:18:19.807 All we do is inject tamoxifen and then we look at what happens to these.

00:18:19.810 --> 00:18:22.650 All we do is inject tamoxifen and then we look at what happens to these.

00:18:22.650 --> 00:18:25.376 we look at what happens to these.

00:18:25.380 --> 00:18:25.734 Phenotypes, and I think you can see from this graph here on the right that within 5 days of treating the mice with tamoxifen, they’re all dead.

00:18:25.734 --> 00:18:28.566 and I think you can see from this graph here on the right that within 5 days of treating the mice with tamoxifen, they’re all dead.

00:18:28.566 --> 00:18:31.326 graph here on the right that within 5 days of treating the mice with tamoxifen, they’re all dead.

00:18:31.326 --> 00:18:34.070 days of treating the mice with tamoxifen, they’re all dead.

00:18:34.070 --> 00:18:35.813 they’re all dead.

00:18:35.813 --> 00:18:36.394 So, so losing MDM two and it’s a
Peachtree dependent process.
Causes this enormous physiological response.
These are some of the pathologies we see in these mice the hippocampus has.
Less number of cells, the retina is comprised of multiple beautiful layers and you can see that it all of these cases that it’s a decreased cell numbers.
There’s some differences in the liver, and it’s actually if you measure liver function.
Liver function is compromised when you have deletions of MDM.
Two kidney has all these protein casts and dilated tubules,
and then in the spleen we have complete absence of white. And as I indicated, these phenotypes are all P53 dependent.

So it just.

I mean, I think the important aspect of this slide is that in some of the previous slide is it highlights some of the pathologies that we might see when we use MDM. Two inhibitors in the clinic to it.

Two inhibitors in the clinic to it. To inhibit the tumors that have high levels of empathy into it. And the hematopoietic defense is actually observed in humans. It is treated with MDM two inhibitors.
But now I want to use this model system to understand what piece of degree is doing in these different tissues. In you know one reason for doing this is we would like to be able to reactivate P53 somehow, and it’s kind of hard people. A lot of people are trying to reactivate people to agree, but what we were hoping is that we might be able to identify downstream pathways to P53. There would be better targets for reactivation tours.
So let me show you what we did.

So again we used our MDM, two conditional mouse and we deleted MDM two in the adult mouse. But we did this acutely and we actually chose a 24 time our time point to ask what P53 targets are regulated in different issues that lead to these pathologies in the adults.

OK, so this is now all the different issues that we initially looked at and what I’m showing you here is the percent recombination. So once we treat with tamoxifen, we induce recombination of the locus.
And you can see that the pancreas, the heart being tested, had the highest level of recombination. I will point out that we only have one MDM, two allele. The other allele is an allele, so single recombination event will activate P53. And then on this axis we chose to look at P53 activation by measuring the expression of P21 which is encodes a cell cycle inhibitor. So you can see in this experiment at the kidney,
the pancreas in the intestine

where the tissues that expressed

the highest levels of P.

And we were thinking the highest

levels of P53.

So we've looked at these mice, so in 24 hours we see no.

Just because P53 is mutant in
Ovarian carcinoma is at 95%. 95% frequency and so we wondered if we might be able to begin to understand that mutation frequency. So for the heart for the ovary, we saw absolutely no phenotypes after 24 hours post deletion of MDM 2. In the intestine we saw a fascinating phenotype which is descript dropout phenotype. In yellow here, I’ve outlined the **** of the intestine and one of the phenotypes is the complete absence of the crypt. Sydney in the lab is quantified.
00:23:08.356 --> 00:23:10.698 the number of **** in these different animals and you can see the mice that have no MDM.
NOTE Confidence: 0.86477065
00:23:10.698 --> 00:23:12.693 2 have about little more than
NOTE Confidence: 0.86477065
00:23:12.693 --> 00:23:15.298 little but half of the number of
NOTE Confidence: 0.86477065
00:23:15.300 --> 00:23:17.586 2 have about little more than
NOTE Confidence: 0.86477065
00:23:17.586 --> 00:23:20.332 little but half of the number of
NOTE Confidence: 0.86477065
00:23:20.332 --> 00:23:24.020 **** is in normal control mouse.
NOTE Confidence: 0.86477065
00:23:24.020 --> 00:23:26.150 The kidney also had some phenotypes
NOTE Confidence: 0.86477065
00:23:26.150 --> 00:23:28.714 at 24 hours and it had twice
NOTE Confidence: 0.86477065
00:23:28.714 --> 00:23:32.300 so you can see here.
NOTE Confidence: 0.86477065
00:23:32.300 --> 00:23:35.540 So this is an early phenotype in the kidney.
NOTE Confidence: 0.86477065
00:23:35.540 --> 00:23:36.012 Again,
NOTE Confidence: 0.86477065
00:23:36.012 --> 00:23:39.316 this is the normal kidney control experiment.
NOTE Confidence: 0.86477065
00:23:39.320 --> 00:23:42.648 And then the pancreas had to be a
NOTE Confidence: 0.86477065
00:23:42.648 --> 00:23:44.345 fascinating phenotype which will
NOTE Confidence: 0.86477065
00:23:44.345 --> 00:23:47.110 delve into a little bit more deeply.
But we saw in the pancreas is this? Acinar to ductal metaplasia, so here’s a normal pancreas in the top, and here’s what the pancreas looks like in the animals that have no MDM. Here we’ve stained with keratin 19, which is a marker for a ductal cell. And here we’ve measured the Metaplastic area and we also see an immune infiltration in these in these mice. So within 24 hours we saw this huge plasticity in the pancreas from you know, this acinar to ductal metaplasia. OK, so we’ve taken these five
tissues an we’ve done.

We’ve looked for expression of P53 targets, so on the left here I show you all of

The dark region is the region that is upregulated in the lighter color, shows the regions that were downregulated in each of these five tissues.

On the right here I show the percent of these dysregulated genes that are actually P53 targets. They have a pizza degree binding site and we used data from the literature to identify these tools.

With P53 binding sites.
So in the intestine, for example, I think that number is 69% of the channels had pizza pre binding sites. So the most. Most of the genes dysregulated it in the system by deletion of MDM 2RP53 targets the. ** the other hand had a huge physiological response, 600 for jeans that were dysregulated but only 16% repeated different targets. So what we’re capturing here at 24 hours is not just you know activation of P53 and P3 targets,
But the downstream responds to P53 activation. So this is now compilation of all those five different tissues to examine the overlap in P53 target genes. And as you can see from this figure on the left there were only 7 jewels that were commonly regulated. By MDM 2 lost that repeat 53 targets. So for example here in the pink is we have 206 genes, 135 of the P53 targets are specific to the pancreas and seven were shared with the other four tissues. These seven jeans are MDM two cycling, G1 MDM two as we mentioned, the very beginning is regulated.
00:26:38.195 --> 00:26:41.189 by P53 and we expect it well.
00:26:41.190 --> 00:26:44.670 We didn’t expect them to be able to,
00:26:44.670 --> 00:26:48.492 but it’s not a surprising result because
00:26:48.492 --> 00:26:51.789 the promoter is intact in MDM 2.
00:26:51.790 --> 00:26:55.446 Three of these genes segment you one GST,
00:26:55.450 --> 00:26:58.570 one piece art, one or cell cycle regulators
00:26:58.570 --> 00:27:01.838 to these jeans are transcription factors,
00:27:01.840 --> 00:27:05.039 and this gene EDA 2R herself directions.
00:27:05.040 --> 00:27:07.638 So these are the six Peachtree
00:27:07.638 --> 00:27:10.070 targets that have a common.
00:27:10.070 --> 00:27:13.297 They represent the common signature of of
00:27:13.297 --> 00:27:16.470 upregulated genes in in these three tissues.
00:27:16.470 --> 00:27:19.314 We wanted to validate the signature
00:27:19.314 --> 00:27:22.199 to make sure that they were.
00:27:22.200 --> 00:27:24.340 Truly, a P53 targets physiologically.
And so what we did is we did our DNA damage ionizing radiation experiment. We treated the whole animal with ionizing radiation, and here's the data for two of the jeans, and we've done it for all of seven cycling G1 E DA2R. Here's the wild type levels of expression of these genes. If we irradiate, you can see that these genes are upregulated. In both cases, and if we irradiate a P53 null, you see you see no up regulation. So these are P3 target genes that are being upregulated following punishing.
NOTE Confidence: 0.80729765
00:28:06.383 --> 00:28:09.930 radiation. So these experiments.
NOTE Confidence: 0.80729765
00:28:09.930 --> 00:28:12.830 Highlight this incredible repertoire.
NOTE Confidence: 0.80729765
00:28:12.830 --> 00:28:15.730 Transcriptional targets that P53
NOTE Confidence: 0.80729765
00:28:15.730 --> 00:28:17.979 physiologically regulates the vivo
NOTE Confidence: 0.80729765
00:28:17.979 --> 00:28:20.513 and I think it also suggests that
NOTE Confidence: 0.80729765
00:28:20.513 --> 00:28:22.676 maybe these specific targets can
NOTE Confidence: 0.80729765
00:28:22.676 --> 00:28:25.256 be used to understand in vivo.
NOTE Confidence: 0.80729765
00:28:25.260 --> 00:28:26.688 If you have.
NOTE Confidence: 0.80729765
00:28:26.688 --> 00:28:30.020 If you can reactivate piece of D3
NOTE Confidence: 0.80729765
00:28:30.127 --> 00:28:33.439 or convert mutant and wild type,
NOTE Confidence: 0.80729765
00:28:33.440 --> 00:28:37.142 these might be great markers to
NOTE Confidence: 0.80729765
00:28:37.142 --> 00:28:40.070 look at for activation 53.
NOTE Confidence: 0.80729765
00:28:40.070 --> 00:28:40.551 OK,
NOTE Confidence: 0.80729765
00:28:40.551 --> 00:28:43.918 I want to now just briefly discuss
NOTE Confidence: 0.80729765
00:28:43.918 --> 00:28:47.090 this this encrypted hypothesis.
NOTE Confidence: 0.80729765
Acinar ductal hyperplasia that we see within 24 hours in the pancreas. So one of the other experiments that Sidney Moyer in my lab did is we obtained these mice MST one missed one. A criar transgene, which means that you can express it only in the pancreas and so Sydney worked out the tamoxifen conditions that gave you a similar percent recombination as
our previous experiments with MDM.

Two position in the entire pancreas.

OK, so similar percentage of recombination and similar activation of P3 targets.

So here we use two of our targets, EADE, DA2RG, STT, SC1.

To measure people to the activation and you can see in both my sweet.

MDM two deletion happens in the home pancreas or mice where it only happens in the acinar cells.

You have similar activation of these three of these targets, so we felt we could do.

We could actually compare deletion
00:30:15.920 --> 00:30:18.450 of MDM two in the whole pancreas. 
NOTE Confidence: 0.7735238
00:30:18.450 --> 00:30:20.982 The deletion of MDM two jestoni 
NOTE Confidence: 0.7735238
00:30:20.982 --> 00:30:21.826 Essen ourselves. 
NOTE Confidence: 0.7735238
00:30:21.830 --> 00:30:25.430 And we have absolutely no phenotype. 
NOTE Confidence: 0.7735238
00:30:25.430 --> 00:30:28.568 So these pancreas look completely normal. 
NOTE Confidence: 0.7735238
00:30:28.570 --> 00:30:32.238 Here we’re measuring just we’re looking at. 
NOTE Confidence: 0.7735238
00:30:32.240 --> 00:30:33.288 I mean, 
NOTE Confidence: 0.7735238
00:30:33.288 --> 00:30:37.480 I ageny sections in here in the right. 
NOTE Confidence: 0.7735238
00:30:37.480 --> 00:30:42.196 component and these these pancreas. 
NOTE Confidence: 0.7735238
00:30:42.200 --> 00:30:46.260 These pancreatic perfectly normal. So. 
NOTE Confidence: 0.7735238
00:30:46.260 --> 00:30:49.487 The take home message here is that. 
NOTE Confidence: 0.7735238
00:30:49.490 --> 00:30:51.700 This esnard ductal hyperplasia that 
NOTE Confidence: 0.7735238
00:30:51.700 --> 00:30:55.830 we see is a P53 specific hyperplasia. 
NOTE Confidence: 0.7735238
00:30:55.830 --> 00:30:58.920 But it’s it’s arising from signals 
NOTE Confidence: 0.7735238
00:30:58.920 --> 00:31:01.630 outside of the acinar cells.
00:31:01.630 --> 00:31:03.487 So to me,

00:31:03.487 --> 00:31:06.582 this is a fascinating experiment

00:31:06.582 --> 00:31:10.507 because no one’s ever noted that.

00:31:10.510 --> 00:31:13.906 That the environment can affect

00:31:13.906 --> 00:31:16.170 the pizza delivery response,

00:31:16.170 --> 00:31:20.790 and so we’ll be delving into understanding

00:31:20.790 --> 00:31:24.898 this phenotype a little bit better.

00:31:24.900 --> 00:31:28.862 Pancreas is one of the tumors

00:31:28.862 --> 00:31:32.180 with 7075% mutations in P53 an and

00:31:32.180 --> 00:31:35.066 it always has this very compromised

00:31:35.066 --> 00:31:38.738 stromal component and so maybe by

00:31:38.738 --> 00:31:43.319 understanding what P 53 is doing is

00:31:43.319 --> 00:31:45.305 physiologically important Organism,

00:31:45.310 --> 00:31:49.377 we might be able to impact our

00:31:49.377 --> 00:31:52.929 understanding of Peter mutations in

00:31:52.932 --> 00:31:55.537
pancreatic cancer. OK, so the let out.

OK, so I'm just going to check my Clock to see how much time I'm doing.

Well, OK, so I've shown you a lot of data where we deleted MDM two and an I didn't show you data for MDM 4 but you see these people three dependent physiological phenotypes and that's all fine and good.

What happens in human cancers is you've got high expressions of MDM two and this is just yes, mean Valentina Vega in the lab.
a number of years ago looked at Indian 2 levels in head and neck squamous carcinomas and these are some of her beautiful pictures.

So here’s MDM, two expressed a very highly in a small region of this squamous cell carcinoma here. 6 expressed almost across the entire tissue and then here is an interesting example of MDM. To be expressed in the cytoplasm, not the nucleus. So we really don’t understand what it’s doing in the cytoplasm, but not in all three of these experiments, P.
00:33:02.033 --> 00:33:03.948 53 is 1 type OK,
NOTE Confidence: 0.7735238
00:33:03.950 --> 00:33:06.866 so I think with this experiment
NOTE Confidence: 0.7735238
00:33:06.866 --> 00:33:08.810 in many others that
NOTE Confidence: 0.8437264
00:33:08.909 --> 00:33:13.180 people have done. Again.
NOTE Confidence: 0.8437264
00:33:13.180 --> 00:33:16.636 Again, show that what MDM two is doing in
NOTE Confidence: 0.8437264
00:33:16.636 --> 00:33:19.967 these tissues is inhibiting P53 activity.
NOTE Confidence: 0.8437264
00:33:19.970 --> 00:33:23.466 Now the I also don’t want to leave
NOTE Confidence: 0.8437264
00:33:23.466 --> 00:33:26.876 you with the notion that MDM too.
NOTE Confidence: 0.8437264
00:33:26.880 --> 00:33:30.760 The P53 is the only MDM to target.
NOTE Confidence: 0.8437264
00:33:30.760 --> 00:33:32.708 Physiologically is the most
NOTE Confidence: 0.8437264
00:33:32.708 --> 00:33:35.630 relevant target because of the cell
NOTE Confidence: 0.8437264
00:33:35.717 --> 00:33:38.037 lethal phenotypes that we see,
NOTE Confidence: 0.8437264
00:33:38.040 --> 00:33:41.603 but in several experiments that my lab
NOTE Confidence: 0.8437264
00:33:41.603 --> 00:33:44.830 and Carol previous labs have done is,
NOTE Confidence: 0.8437264
00:33:44.830 --> 00:33:47.700 we’ve tried to overexpress MDM two in
NOTE Confidence: 0.8437264
00:33:47.700 --> 00:33:50.613 normal cells to understand what it’s
NOTE Confidence: 0.8437264
00:33:50.613 --> 00:33:53.817 actually doing in with regards to transformation and tumor evolution.
NOTE Confidence: 0.8437264
00:33:55.980 --> 00:33:57.788 So here’s what happens.
NOTE Confidence: 0.8437264
00:33:57.788 --> 00:34:01.501 So this is a normal control and the
NOTE Confidence: 0.8437264
00:34:01.501 --> 00:34:04.665 left these are mouse cells express a
NOTE Confidence: 0.8437264
00:34:04.665 --> 00:34:07.790 normal number of mouse chroma zones,
NOTE Confidence: 0.8437264
00:34:07.790 --> 00:34:11.042 and when we overexpressed MDM two
NOTE Confidence: 0.8437264
00:34:11.042 --> 00:34:14.180 we see this incredibly abnormal.
NOTE Confidence: 0.8437264
00:34:14.180 --> 00:34:16.655 Chromosome instability we can quantify the numbers of fusions here and
NOTE Confidence: 0.8437264
00:34:16.655 --> 00:34:19.558 we have a huge number of fusions.
NOTE Confidence: 0.8437264
00:34:21.990 --> 00:34:26.645 We also have a lot of fragments.
NOTE Confidence: 0.8437264
00:34:26.650 --> 00:34:29.140 So in data from multiple labs,
NOTE Confidence: 0.8437264
00:34:29.140 --> 00:34:31.954 if you overexpress MDM two in a
NOTE Confidence: 0.8437264
00:34:31.954 --> 00:34:34.538 normal cell the cell just dies.
NOTE Confidence: 0.8437264
It can continue to grow.

So and you know some experiments that are ongoing in the lab is OK,

OK, so if we can’t overexpress MDM two in a normal cell?

Why do tumors have very high levels of ambient 2IN in one idea that we’re working with is that there are some other rotation in those tumors that allows those tumors to survive with high levels of MDM two and so if we could understand what else MDM two is doing, we might be able to obtain a window of vulnerability to try to get the
MDM two overexpressing cells to implode.

But the screens that we’re doing currently are ongoing.

OK then for the last few minutes of my lecture I want to.

Move over to our understanding of P53 mutations in breast cancer models.

Didn’t tell you earlier, but pizza degree.

Why did tell you the people limitations for the most common, but really it speak into three missense mutations that are the most common type of genetic lesion, and so my lab and that of Tyler left.

Tyler Jacks is lab have made germline
mutations in P53IN animal models and we show that these mice are tumor pro. But more importantly in contrast. Green or mice? These mice have a high metastatic capability, so this here is our data from the 172 mutation corresponds to the origin 175 mutation, which is one of the hot spot mutations in human cancers. And here you can see a metastasis to the liver, and here stained with the P53 antibody, a metastasis to the brain. And this is in contrast to mice that have deletions of 353,
so this really was the first example that suggested that expressing a mutant P53 was much more aggressive than not having people to create and in the field. We call this a gain of function. Mutant P53 is doing something in these cells to make them highly metastatic. So these are germline mice and what we wanted to do is to generate semantic models because these germline models represent Lee from Many syndrome which is an inheritance of people to mutations. But that’s a rare syndrome and we really wanted to understand this metastatic
phenotype in a system where the the specific cell type has a peach limitation and surrounding normal environment. To feel yourself to catch neutral goes to T cells are all wild type for P53 so that mouse did not exist. Tyler Jacks made a beautiful mouse that has been used extensively in the literature that basically is heterozygous for P53. So the entire mouse is missing 1P53 allele and Natalie all can be converted to a mutant P53 in a tissue specific fashion. So we didn’t think that that was. Adequate enough to study the tumor, stroma tumor immune interactions because of heterozygosity of the P53 locus.
So let me tell you a little bit about how we generate these mice and what our breast tumor phenotype is.

OK, so here’s how we generated these alleles. So we call these WM allele for wild type to mute P53.

So I’ll show you in a minute this is a wild type allele normally and this example is the Argentine, once it imitation which we generated previously. So what you have is a wild type P50.
Three years of pollination site and then in a cream immediate fashion you can remove the wild type C DNA and basically reconstruct the locus. With these mice and because it took a very long time to make these animals, we actually decided to make a second hotspot mutation and that’s the Argentine 2.5 to double mutation, which corresponds to the 248 hotspot mutation.
You make them into protein and you can make it in an 
aksri dependent manner. So this just shows you how normal 
those mice are.

So here we're comparing wild type 2 heterozygous mice with the 172 or the 2.5. 
We stabilized the mutant protein in response to DNA damage. 
And when we look at the activation of the three targets be 21 in Puma, 
they were activated to similar levels. 
We looked at the ability of these. 
Of DNA damage to induce labor ptosis? No difference between these 
two alleles in wild type mice.
And again for the ability to rest the cycle in mouse embryo fibroblasts, there's no difference, so these mutant alleles really represent these condition alleles.

That column you Tilly's really represent wild type allele. They can become a moot.

What we've done is what we've done here is compared the 172 heterozygous mice at 245 headers. I guess mice and wild type mice to each other over more than two years to look at the tumor phenotypes.
Mice is the age.

Just like people will sporadically get tumors, but what you can see is that there's absolutely no statistical difference between the three alleles. So for all practical purposes.

This new allele that we generated expresses a wild type P53 protein.

OK, so let me tell you about two experiments. One is our semantic breast semantic model and what we did is we injected Adna virus Cree into the duct of the mammary gland and so and then. In addition, we use this TV tomato.

NOTE Confidence: 0.749259
allele which is also create dependent.

So when we inject adeno Korea not show you a picture in a minute.

We basically make a mutant P and we label the cell red.

Control experiments to show that we do get recombination when we inject the cream expressing adeno virus here. On the left is a low titer injection and you can see the red cells here. A high titer virus was used was injected into this gland in about 50 to 70% of the ductal cells are checked.

1 to 5% of the ductal cells become infected.
I also want to note that these mice are 50% balzi. Normally the P53 field is used to see 57 black, 6 string to study. Peabody create tumor phenotypes, but that strain is resistant to breast cancers for some reason, and the beltsy component brings in more sensitivity to breast cancers, and we really don’t know the genetic reasons for this.

OK, so here is the data. Let me go through it in detail. So this is the 172 mutation expressed only in a few mammary glands.
Memory cells, low titer.
We didn’t see any tumors.
High titer, we actually didn’t see any tumors.
This one tumor showed up.
Post that to your end time point of the experiment.
Because we weren’t sure we were going to get any tumors by just making people scream Mutant in a few cells.
Irradiated with the sub lethal dose of radiation.
This is not lethal to the mouse, but it does cause damage and if the damage is in the right or wrong genes
that contributes to tumor phenotype.

So with a low titer we begin to see tumors.

We sell one at a higher titer.

We now solve 4 tumors and one tumor metastasized.

The 2.5 allele was a much stronger, had a much stronger tumor,
phenotype with low titer.

We saw four tumors and one of them was meta static with the high tier tighter.

We saw nine tumors, so this is about 75% and more than half were meta static.

So let me kind of summarize all the data we’ve done with these animals.

So first let’s just look at
the tumor incidence. The R 172.

In these experiments, we only use one copy because we didn’t want to. We didn’t want to do LOH and we just wanted to figure out what would happen with the minimal number of alterations.

So if you compare the ones need two hitters Vegas to the 2.5 low and high titer, there’s a huge number of tumors in the 248.

Nice if we irradiated the ones only two, we got increased tumor incidence and then this is the experiment where we did mutate the other allele.
So existing law supporters I got city and we can see. Increased tumor phenotype with the high dose. And this is a comparison on in the middle panel of the metastatic phenotype, and again the 245 documentation was the most metastatic. And then we looked at lots of header zygosity so the 245 mutation had. Variations in terms of LOH. About 50% of the mice showed LOH and then others retained some or all of the P3 alleles. The irradiated are once again showed 100% LOH so to us.
While we don’t understand why we see 100% outrage with this allele, would it says? Is it that wild type allele is very strong at inhibiting tumors in this winsome need to background? These are the breast tumor subtypes that we saw the irradiated once in need two with the 2.5 mutation we saw all three. Molecular subtypes to me this is fascinating experiment because we’ve made one mutation, we made a P53 mutation and yet here in this
00:46:23.941 --> 00:46:28.180 sample with the 2:45 we see off the tumor,
00:46:28.180 --> 00:46:31.484 molecular subtypes evolving and so one of
00:46:31.484 --> 00:46:34.592 the experiments that we’re doing now is
00:46:34.592 --> 00:46:37.780 trying to understand with this 245 mutation,
00:46:37.780 --> 00:46:40.065 what are the triggers to
00:46:40.065 --> 00:46:41.436 these different subtypes?
00:46:41.440 --> 00:46:43.408 Triple negative breast cancer
00:46:43.408 --> 00:46:45.868 is very hard to treat.
00:46:45.870 --> 00:46:47.139 But for example,
00:46:47.139 --> 00:46:49.677 here to enrich tumors you can,
00:46:49.680 --> 00:46:52.634 you can treat with her two antibodies,
00:46:52.640 --> 00:46:54.765 so we’re trying to understand
00:46:54.765 --> 00:46:56.465 basically the tumor evolution
00:46:56.465 --> 00:46:59.355 that initiates with this one, P.
00:46:59.355 --> 00:47:01.740 53 missense mutation.
We've also wanted to so the data. I just showed you says that the 248 mutation is much more. Dramatic. Then the 175 mutation. So what I showed here is a comparison of ovarian lung and breast tumors from people and just looking at the kind of mutation that they have and you can see in people that the 248 mutation is has the worst outcomes. We couldn't do these data just for breast because the number of samples out there was not enough to give us significance.
so the one of the last experiments I’ll show you here is just trying to understand tumor evolution because we made a semantic model that develop different kinds of breast cancers that were highly metastatic. And so I’m really interested in understanding the task sees in an in vivo physiologically relevant system. So we did. Here is we took the 2:45 mutant animals and we took. UH-22 memory tumors from these mice we sequenced them in three different regions trying to understand a
little bit about the heterogeneity, and then we sequenced three metastases from each of these tumors. OK, and we sequence them to an incredible depth. So what we have here then is the comparison of the primary to the metastases and if we just look at mouse #4, there is some overlap in these these this overlap is considered an early gene signature and then you see this. Slate sequences that come up, which is how, and which is what, where the metastases is now evolving.
when it gets into its metastatic site, which in this case was the lung. And so we can compare the early mutations of all three. The task season you see early mutations in both experiments, so these are the mutations that were acquired in the primary and metastatic lesion at the same time. But when you look at late mutations, here’s all three mutations for mouse #4 and there’s only one late mutation common. So with these sequencing data, indicate is that these matasa fees left the tumor very early.
00:49:38.039 --> 00:49:40.574 during the metastatic process and
NOTE Confidence: 0.7825957
00:49:40.574 --> 00:49:42.602 then seated and had
NOTE Confidence: 0.7901794
00:49:42.695 --> 00:49:46.722 additional changes. So this was the
NOTE Confidence: 0.7901794
00:49:46.722 --> 00:49:49.712 first suggestion that maybe metastasis.
NOTE Confidence: 0.7901794
00:49:49.720 --> 00:49:51.632 Breast cancer metastasis driven
NOTE Confidence: 0.7901794
00:49:51.632 --> 00:49:55.749 by a new P53 is an early event.
NOTE Confidence: 0.7901794
00:49:55.750 --> 00:49:58.035 So to summarize, this model
NOTE Confidence: 0.7901794
00:49:58.035 --> 00:50:00.351 just briefly, we can make.
NOTE Confidence: 0.7901794
00:50:00.351 --> 00:50:04.303 P 3 point mutation in just a few
NOTE Confidence: 0.7901794
00:50:04.303 --> 00:50:07.717 cells that become a tumor that.
NOTE Confidence: 0.7901794
00:50:07.720 --> 00:50:09.502 Interessee migrate,
NOTE Confidence: 0.7901794
00:50:09.502 --> 00:50:13.957 proliferate and develop these metastases.
NOTE Confidence: 0.7901794
00:50:13.960 --> 00:50:16.312 Where we now have I called it a
NOTE Confidence: 0.7901794
00:50:16.312 --> 00:50:18.389 little factory but we just have
NOTE Confidence: 0.7901794
00:50:18.389 --> 00:50:20.164 these mice now developing tumors.
NOTE Confidence: 0.7901794
00:50:20.170 --> 00:50:22.459 We can isolate the circulating tumor cells,
so we're trying to do is understand that
the changes that occur for these cells
to be able to survive in the blood.
To home into an organ and then to develop.
OK, if I have a few minutes which
I have just a few minutes,
I’m going to tell you about the
other model that we made because we
weren’t sure that making a people
communication in just a few cells was
going to give us a tumor phenotype.
So here we use K14 create,
which expresses then a mutant P53 in all
of the epithelial cells of the mammary gland,
and this is a model that develops.
00:51:02.310 --> 00:51:03.582 Triple negative breast cancer 100% of the time.
NOTE Confidence: 0.7901794
00:51:06.956 So again this.
NOTE Confidence: 0.7901794
00:51:09.061 This suggests that you know South normal cell tumor cell interactions are altering the kinds of tumors that come up.
NOTE Confidence: 0.7901794
00:51:22.720 And in this scenario we also had a cast 9 allele that is Creed dependent,
NOTE Confidence: 0.7901794
00:51:26.131 so K14 CRV and allows us to make a mutant people degree in just a few in the epithelium of the mammary gland and to express castanon so we can use CRISPR technologies to to begin to address vulnerabilities.
NOTE Confidence: 0.7901794
00:51:40.819 OK, so this vulnerability that we examine
this in this model was whether these tumors were addicted to having immune to P53.

OK, so here is the use that adnot associated virus that expresses a guide RNA that will delete P.

So the tumor burden before treatment with a V in the control and the mute. Depleted tumors with similar,

but you can see here in in the Purple line that those tumors that had depletion of you piece of degree survived much longer.

This is just a picture of the tumor phenotypes that control you can see that.
It’s very.

It’s obviously a tumor.

These mice die very quickly post identification of the tumors, and then you can see here with the depletion immunity theory this this gland is looking more normal.

OK, this is a whole bunch of data for the individual mice that this is a tumor volume. The controls and green here.

Once we identify the tumor, they just keep growing the experimental cohort here in purple are animals that have recombined have basically deleted that mute people free protein.
00:53:05.827 --> 00:53:08.167 In these mice, live much longer.

00:53:08.170 --> 00:53:11.138 On the right is a tumor volume,

00:53:11.140 --> 00:53:14.458 so you can see that depletion of

00:53:14.458 --> 00:53:17.269 P53 affects the tumor volume.

00:53:17.270 --> 00:53:19.730 Greatly and then I'll just point

00:53:19.730 --> 00:53:22.210 out these two samples in Orange,

00:53:22.210 --> 00:53:24.320 which appeared not to respond

00:53:24.320 --> 00:53:26.430 to depletion of Mutant P

00:53:26.515 --> 00:53:28.810 and when we look at

00:53:28.810 --> 00:53:30.870 these two samples in detail,

00:53:30.870 --> 00:53:34.158 they did not express a stable mutant P.

00:53:34.160 --> 00:53:37.154 and so we think that

00:53:37.154 --> 00:53:40.260 these two tumors are actually.

00:53:40.260 --> 00:53:42.306 Since the P 53 isn't stable,

00:53:42.310 --> 00:53:44.058 the evolution that's occurring
in these tumors is due to the absence of the P53 protein and not to a gain of function.

OK, so I'm going to stop there and just I thrown a lot of data at you so I wanted to summarize briefly the first set of experiments really captured the exquisite sentence sensitivity of the MDM, two protein and P53 activity. We were able to identify this the molecular response to P53 activation in vivo identified numerous targets that are tissue specific. What are they all doing? I think we have our work cut out.
for us 'cause there's no way I can delete every one of these targets and see how important they are in vivo and then last but not least, I showed you the generation of this novel mouse model that really allows us now to make a semantic point potential 53 in. Any cell of origin that we want to an in the breast model. Specifically, we had a highly metastatic phenotype that we’re trying to understand in more detail. OK, so my last slide is just the
numerous people in the lab that have contributed to the studies. The Vinodh Pant did the feedback loop studies Roberto Navy MTM Tunicate Johnny DMD. Or not, God Tamera did all the studies with the radiation and with the node to look at the P3 response, Sidney generated the conditional mouse to look at the acute activation of people. Three targets you in terrific postdoc in the lab now has her own independent position generated that conditional mood P53 allele and Donata is the one who’s studying the addiction.
00:55:42.120 --> 00:55:44.135 We're really wondering what the
mechanisms is acquire these
NOTE Confidence: 0.8528144
00:55:44.135 --> 00:55:45.747 tumors imploding when they no
NOTE Confidence: 0.8528144
00:55:45.747 --> 00:55:47.919 longer have communities industry.
NOTE Confidence: 0.8528144
00:55:47.919 --> 00:55:49.627 So with that, I'll end in, oh,
NOTE Confidence: 0.8528144
00:55:49.630 --> 00:55:52.742 I'm glad to answer any questions.
NOTE Confidence: 0.8528144
00:55:52.742 --> 00:55:56.414 Thank you so much.
NOTE Confidence: 0.8138522
00:55:56.820 --> 00:55:58.011 That was a wonderful talk.
NOTE Confidence: 0.8138522
00:56:00.000 --> 00:56:02.835 I'm going to ask people to put
NOTE Confidence: 0.8138522
00:56:02.835 --> 00:56:05.179 questions in the chat,
NOTE Confidence: 0.8138522
00:56:05.180 --> 00:56:08.756 but I I wanted to ask you, sort of,
NOTE Confidence: 0.8138522
00:56:08.756 --> 00:56:10.746 from the therapeutics perspective,
NOTE Confidence: 0.8138522
00:56:10.750 --> 00:56:13.246 people have been very interested in
NOTE Confidence: 0.8138522
00:56:13.246 --> 00:56:15.637 compounds like Prima and Cody that
NOTE Confidence: 0.8138522
00:56:15.637 --> 00:56:18.245 assist with re folding of P53 with
disruptive mutation and yet clinically those have been a little bit disappointing. Is much known about whether or not those refolded P 53's are better, worse the same as substrates for the MDM? Two MDM four? Yeah, so, so we’ve done a few studies using some of the drugs that are available, not many. My lab is focused on the genetics because if we take out him to an MP4 we see people three different phenotypes, but you know it’s very different. Genetic told us mechanisms, but the drugs are really as you indicated. They’re going to tell us
whether they work or not.

So I agree with you, I don’t think drugs are working very well.

And you know, I don’t know enough about those experiments to know how often the drug with the level of activation.

That gene signature that we identified would really help in those studies to try to understand what is the pika degree response?

I also think that the people agree response required.
Will vary in different tissues. We just know from our MDM two studies that some tissues are just much more sensitive to increase P 53 levels versus others. So I think that there's just a whole lot more work to be in to do in the clinic to be able to understand that response. Yeah, I think having a common set of. I mean I think people have just looked over and over again and it's probably very inadequate, right? I see a couple questions and we have like a minute left. So first Jeff Townsend wants to know whether or not you've considered
looking at sequencing of much larger cohorts of tumors and multi sample datasets to understand the temporal order of mutation appearances, that’s exactly what we’re doing right now I have a postdoc in a graduate student who just generated. A cohort of 100. Nice ‘cause we want to understand the sequence of events. We want to understand the different events that lead to the different molecular subtypes. So we are in the midst of those experiments and we’re going to do RNA sequencing to understand both what happens if Arnie level.
I think it’s critical we need to understand what’s happening at the DNA level because I think that’s what gives rise to the different molecular subtypes. But I think it’s the expression that’s really going to tell us what’s happening to those cells once they reach home to deliver the line so? So we got all those are in progress. Then, Karen Anderson, who’s my Co. Host for having invited you, wants to ask what your thoughts might be for a pro TEC directed against MDM two try to grade that.
as a therapeutic strategy. I think there’s two Proteins we should be thinking about. One is MDM 2. Although, somehow I think for an MDM two inhibitor I think you’ve got to target it to the tumor cell. Better just because of the hematopoetic toxic cities that have been seen with the MTM 2 inhibitors. But I also think we should start thinking about potentially doing degrading mutant P.
Particularly with those gain of function mutations, exactly.

We are over there more questions but we are over the time so I want to be respectful of very appreciative of you, having joined us today and if anybody wants to ask me a question, email that they should feel free to I think allowed to answer additional questions.

Super very much.