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 P.53 works as a transcriptional activator. Many, many findings regarding the ways that MDM two and MDM three regulate P.53. 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.
00:01:22.620 --> 00:01:25.105 She is now chair of the Department of Genetics and Hubert L. Olive Stringer, distinguished chair in oncology.

00:01:28.042 --> 00:01:30.366 In the division of Basic Science Research at MD Anderson,

00:01:32.278 --> 00:01:36.043 so I don't want to talk for a long time because I'm really eager.

00:01:37.785 --> 00:01:40.230 to hear what you have to say.

00:01:42.590 --> 00:01:45.320 and I will just say to the audience.

00:01:47.714 --> 00:01:49.450 enter your questions or we can.

00:01:51.394 --> 00:01:53.579 at the end if we need to.

00:01:55.170 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
the P53 tumor suppressor pathway.

NOTE Confidence: 0.83147156

So I’ll get started with my disclosures.

NOTE Confidence: 0.83147156

I am on the Scientific Advisory Board for PMV Pharma.

NOTE Confidence: 0.83147156

So the P 53 pathway.

NOTE Confidence: 0.83147156

This is my myopic view of the pathway

NOTE Confidence: 0.83147156

and it really highlights.

NOTE Confidence: 0.83147156

I think some of the critical

NOTE Confidence: 0.83147156

features of the pathway.

NOTE Confidence: 0.83147156

First and foremost people decree is present

NOTE Confidence: 0.83147156

in very low levels in normal cells,

NOTE Confidence: 0.83147156

but any kind of abnormality

NOTE Confidence: 0.83147156

that the South senses,

NOTE Confidence: 0.83147156

hypoxia, DNA damage,

NOTE Confidence: 0.83147156

inappropriate activation of an

NOTE Confidence: 0.83147156

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 an inhibitor of the cell cycle. It also induces senescence program. P53 can activate a slew of genes involved in changing the metabolic functions of the cell. If the cell is allowed to survive. Now when P 53 is activated, it also activates.
and proceed,

P53 has to activate this protein

called MDM 2,

which is an E3 ubiquitin ligase

that targets P53 for degradation

and basically removes the Peachtree

levels back down to normal.

So if you just.

Think about what people think we can do.

We can do so much in getting arrested,

and it can also induce its own

inhibitor to allow the cell to survive.

And even though there’s so much

work in the pizza tree killed,

we still don’t understand all of the
NOTE Confidence: 0.83147156

00:04:26.952 -- 00:04:29.238 cues that determine which of these
NOTE Confidence: 0.83147156

00:04:29.238 -- 00:04:31.208 pathways Peabody Creek and activate.
NOTE Confidence: 0.83147156

00:04:31.210 -- 00:04:33.536 But because it has all these functions,
NOTE Confidence: 0.83147156

00:04:33.536 -- 00:04:35.528 it is a critical tumor suppressor,
NOTE Confidence: 0.83147156

00:04:35.530 -- 00:04:37.858 and it is the most doctor
NOTE Confidence: 0.83147156

00:04:37.858 -- 00:04:39.410 mutated gene human cancers.
NOTE Confidence: 0.83147156

00:04:39.410 -- 00:04:42.274 So what I showed here is what the
NOTE Confidence: 0.83147156

00:04:42.274 -- 00:04:44.559 field called the Manhattan Plot,
NOTE Confidence: 0.83147156

00:04:44.560 -- 00:04:47.325 and it was developed by Magali Olivia.
NOTE Confidence: 0.83147156

00:04:47.330 -- 00:04:49.906 So across this axis are 125 genes
NOTE Confidence: 0.83147156

00:04:49.906 -- 00:04:52.258 that are commonly mutated and cancers
NOTE Confidence: 0.83147156

00:04:52.258 -- 00:04:54.988 and then across this axis here are
NOTE Confidence: 0.83147156

00:04:55.062 -- 00:04:57.227 36 different types of cancers,
NOTE Confidence: 0.83147156

00:04:57.230 -- 00:04:59.995 and there’s some features that stand out.
NOTE Confidence: 0.83147156

00:05:00.000 -- 00:05:02.920 But this is the one I want to
NOTE Confidence: 0.83147156

8
highlight here across the board.
NOTE Confidence: 0.83147156

These are mutations in the P53 tumor suppressor.
NOTE Confidence: 0.83147156

So almost all cancers mutate.
NOTE Confidence: 0.83147156

53 but P 53 pathway is inactivated by
NOTE Confidence: 0.83147156

multiple mechanisms and I show here
NOTE Confidence: 0.83147156

in some of the different cancers and
NOTE Confidence: 0.83147156

how they inactivate piece of debris.
NOTE Confidence: 0.83147156

So high grade,
NOTE Confidence: 0.83147156

serious ovarian carcinomas,
NOTE Confidence: 0.83147156

mutations in P53 are the most common.
NOTE Confidence: 0.83147156

However,
NOTE Confidence: 0.83147156

in liposarcomas it is upregulation of MDM 2,
NOTE Confidence: 0.7966159

the P fifty 383 if it could be like a San.
NOTE Confidence: 0.7966159

About 100% of these liposarcomas inactivate
NOTE Confidence: 0.7966159

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 in 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 have neither mutations and piece of D3 or 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 of these proteins that I will discuss with you today.

So MDM two is an inhibitor of P53. It's an E3 ubiquitin ligase and
NOTE Confidence: 0.7966159
00:07:01.786 --> 00:07:03.350 target speakers for degradation.
NOTE Confidence: 0.7966159
00:07:03.350 --> 00:07:07.130 MDM Four also inhibits P 53.
NOTE Confidence: 0.7966159
00:07:07.130 --> 00:07:09.888 It doesn’t have any E3 ligase function,
NOTE Confidence: 0.7966159
00:07:09.890 --> 00:07:12.110 but it actually facilitates and makes
NOTE Confidence: 0.7966159
00:07:12.110 --> 00:07:15.010 MDM two or better yet riveting ligase,
NOTE Confidence: 0.7966159
00:07:15.010 --> 00:07:17.100 although it also has independent
NOTE Confidence: 0.7966159
00:07:17.100 --> 00:07:19.735 functions of MDM two and can
NOTE Confidence: 0.7966159
00:07:19.735 --> 00:07:22.546 actually bind and inhibit the pizza
NOTE Confidence: 0.7966159
00:07:22.549 --> 00:07:24.550 guy free transactivation domain.
NOTE Confidence: 0.7966159
00:07:24.550 --> 00:07:27.546 This relationship does MDM 2 New Four
NOTE Confidence: 0.7966159
00:07:27.546 --> 00:07:30.280 also conform hetero dimer and that
NOTE Confidence: 0.7966159
00:07:30.280 --> 00:07:33.028 header dimer is critical in embryo
NOTE Confidence: 0.7966159
00:07:33.028 --> 00:07:35.440 development to inhibit P 53 and then,
NOTE Confidence: 0.7966159
00:07:35.440 --> 00:07:36.700 as I indicated,
NOTE Confidence: 0.7966159
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
NOTE Confidence: 0.79513806
00:09:42.627 --> 00:09:44.765 important interaction was and what
NOTE Confidence: 0.79513806
00:09:44.765 --> 00:09:46.877 we did was test the importance
NOTE Confidence: 0.79513806
00:09:46.877 --> 00:09:49.608 of P53 in this little embryo by
NOTE Confidence: 0.79513806
00:09:49.608 --> 00:09:52.136 crossing 2P53 miles and we completely
NOTE Confidence: 0.79513806
00:09:52.136 --> 00:09:53.864 rescue this phenotype.
NOTE Confidence: 0.79513806
00:09:53.864 --> 00:09:56.744 These Meister born and are
NOTE Confidence: 0.79513806
00:09:56.744 --> 00:09:59.058 perfectly normal except now because
NOTE Confidence: 0.79513806
00:09:59.058 --> 00:10:01.860 they lack P 53 they have it.
NOTE Confidence: 0.79513806
00:10:01.860 --> 00:10:04.476 So with with this experiment indicates
NOTE Confidence: 0.79513806
00:10:04.476 --> 00:10:08.072 is that what MDM two is doing in
NOTE Confidence: 0.79513806
00:10:08.072 --> 00:10:10.277 these embryos is upregulating P53,
NOTE Confidence: 0.79513806
00:10:10.280 --> 00:10:12.490 which is preventing the normal
NOTE Confidence: 0.79513806
00:10:12.490 --> 00:10:14.258 development of these embryos.
NOTE Confidence: 0.79513806
00:10:14.260 --> 00:10:16.480 MDM fours are related MDM,
NOTE Confidence: 0.79513806
00:10:16.480 --> 00:10:19.138 two protein that aren’t Johansson discovered.
NOTE Confidence: 0.79513806
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 but again that phenotype is rescued by deletion of P53 and we’ve also made mice that have no MDM to know that have Pizza 3 two or phenotypes because they lack P 53. So at least physiologically, these empty in proteins is to keep viable in the mouse,
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 in regulating P53 levers? OK, 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. The mice are fine, but again, most was perfectly normal. We really thought that this feedback loop is going to be critical for regulation of P53.
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. So what is the phenotype? These animals are actually dying because of the complete ablation of the ball mirror, 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 MDM two 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 P. 21, 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 MDM2 for survival.

And.

And if you have too much and you have too much MDM for deletion of 53 deletion of downstream effectors of P53 can rescue those lethal phenotypes.

Now come. Because.

MDM2 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 MDM2 in different tissues at different times.

and we’ve used, we’ve generated this conditional allele of MDM2 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. 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
used a Cree transgene that is tamoxifen inducible so this is this is a mouse that has one of the conditional alleles and it has the other legalism missing, so it’s single recombination event is going to create an M2 normals or not sell in. All we do is inject tamoxifen and then we look at what happens to these. 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. 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 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.
00:20:03.510 --> 00:20:03.854 Uhm?

00:20:03.854 --> 00:20:06.950 But now I want to use this model system to understand what piece of degree is doing in these different tissues.

00:20:09.465 --> 00:20:12.579 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.

00:20:14.974 --> 00:20:17.777 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.

00:20:19.900 --> 00:20:22.210 and it’s kind of hard people.

00:20:22.210 --> 00:20:24.905 A lot of people are trying to reactivate people to agree.

00:20:24.905 --> 00:20:26.829 but what we were hoping is that we might be able to identify downstream pathways to P53.

00:20:26.830 --> 00:20:29.063 There would be better targets for reactivation tours.
00:20:37.340 --> 00:20:40.916 So let me show you what we did.
NOTE Confidence: 0.8166339
00:20:40.920 --> 00:20:43.608 So again we used our MDM,
NOTE Confidence: 0.8166339
00:20:43.610 --> 00:20:45.750 two conditional mouse and we
NOTE Confidence: 0.8166339
00:20:45.750 --> 00:20:48.989 deleted MDM two in the adult mouse.
NOTE Confidence: 0.8166339
00:20:48.990 --> 00:20:52.166 But we did this acutely and we actually
NOTE Confidence: 0.8166339
00:20:52.166 --> 00:20:55.375 chose a 24 time our time point to
NOTE Confidence: 0.8166339
00:20:55.375 --> 00:20:58.102 ask what P53 targets are regulated
NOTE Confidence: 0.8166339
00:20:58.102 --> 00:21:01.348 in different issues that lead to
NOTE Confidence: 0.8166339
00:21:01.348 --> 00:21:05.669 these pathologies in the adults.
NOTE Confidence: 0.8166339
00:21:05.669 --> 00:21:08.112 so this is now all the different
NOTE Confidence: 0.8166339
00:21:08.112 --> 00:21:10.029 issues that we initially looked
NOTE Confidence: 0.8166339
00:21:10.029 --> 00:21:12.682 at and what I'm showing you here
NOTE Confidence: 0.8166339
00:21:12.756 --> 00:21:14.868 is the percent recombination.
NOTE Confidence: 0.8166339
00:21:14.870 --> 00:21:17.258 So once we treat with tamoxifen,
NOTE Confidence: 0.8166339
And you can see that the pancreas, the heart being tested, had the highest level of recombination. Again, 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 encoded by a cell cycle inhibitor. So you can see in this experiment at the kidney.
00:21:51.480 --> 00:21:53.550 the pancreas in the intestine
NOTE Confidence: 0.8166339
00:21:53.550 --> 00:21:55.620 where the tissues that expressed
NOTE Confidence: 0.8166339
00:21:55.687 --> 00:21:57.287 the highest levels of P.
NOTE Confidence: 0.8166339
00:21:57.290 --> 00:21:57.898 21.
NOTE Confidence: 0.8166339
00:21:57.898 --> 00:22:01.546 And we were thinking the highest
NOTE Confidence: 0.8166339
00:22:01.546 --> 00:22:04.000 levels of of P53.
NOTE Confidence: 0.8166339
00:22:04.000 --> 00:22:06.317 So we’ve we’ve looked at these mice,
NOTE Confidence: 0.8166339
00:22:06.320 --> 00:22:08.630 so in 24 hours we see no.
NOTE Confidence: 0.8166339
00:22:08.630 --> 00:22:11.609 So let me back up just for one second,
NOTE Confidence: 0.8166339
00:22:11.610 --> 00:22:13.596 I hope I can do that,
NOTE Confidence: 0.8166339
00:22:13.600 --> 00:22:15.180 so that issues that we
NOTE Confidence: 0.8166339
00:22:15.180 --> 00:22:16.760 decided to look at where
NOTE Confidence: 0.84411275
00:22:16.830 --> 00:22:18.432 the kidney, the pancreas,
NOTE Confidence: 0.84411275
00:22:18.432 --> 00:22:20.587 the intestine in the heart.
NOTE Confidence: 0.84411275
00:22:20.590 --> 00:22:22.876 And the ovary in the ovary.
NOTE Confidence: 0.84411275
00:22:22.880 --> 00:22:25.652 Just because P 53 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.
the number of **** in these different animals and you can see the mice that have no MDM. 2 have about little more than little but half of the number of **** is in normal control mouse. The kidney also had some phenotypes at 24 hours and it had twice the number of protein casts, so you can see here. So this is an early phenotype in the kidney. And then the pancreas had to be a fascinating phenotype which will 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 and we’ve done.

We’ve looked for expression of P53 targets, so on the left here I show you all of the genes that were regulated in these different issues in our RNA seek data. 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 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 that 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.

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.

So these seven jeans are MDM2 cycling, G1 MDM2 as we as I 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 in both cases, and if we irradiate a P53 null, you see no up regulation.

So these are P3 target genes that are being upregulated following punishing
radiation. So these experiments.

Highlight this incredible repertoire.

Transcriptional targets that P53 physiologically regulates the vivo and I think it also suggests that maybe these specific targets can be used to understand in vivo. If you have. If you can reactivate piece of D3 or convert mutant and wild type, these might be great markers to look at for activation 53. OK, I want to now just briefly discuss this encrypted hypothesis.
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. One is an SNR specific transcription factor. An upstream of the missed one promoter. There is a criar transgene, which means that you can express create 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
of MDM two in the whole pancreas.

The deletion of MDM two just

So these pancreas look completely normal.

Here we’re measuring just we’re looking at.

I mean,

I ageny sections in here in the right.

We’re measuring the immune

component and these these pancreas.

These pancreatic perfectly normal. So.

The take home message here is that.

This esnard ductal hyperplasia that

we see is a P53 specific hyperplasia.

But it’s it’s arising from signals

outside of the acinar cells.
So to me, this is a fascinating experiment because no one’s ever noted that. That the environment can affect the pizza delivery response, and so we’ll be delving into understanding this phenotype a little bit better. Pancreas is one of the tumors with 7075% mutations in P53 and it always has this very compromised stromal component and so maybe by understanding what P 53 is doing is physiologically important Organism, we might be able to impact our understanding of Peter mutations in...
pancreatic cancer. OK, so let’s get 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 you see these people three

dependent physiological phenotypes

It showed us how important this

relationship is between these proteins but.

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
NOTE Confidence: 0.7735238
00:32:29.294 --> 00:32:31.597 a number of years ago looked at
NOTE Confidence: 0.7735238
00:32:31.597 --> 00:32:33.774 Indian 2 levels in head and neck
NOTE Confidence: 0.7735238
00:32:33.843 --> 00:32:36.038 squamous carcinomas and these are
NOTE Confidence: 0.7735238
00:32:36.038 --> 00:32:38.233 some of her beautiful pictures.
NOTE Confidence: 0.7735238
00:32:38.240 --> 00:32:39.179 So here’s MDM,
NOTE Confidence: 0.7735238
00:32:39.179 --> 00:32:41.860 two expressed a very highly in a small
NOTE Confidence: 0.7735238
00:32:41.860 --> 00:32:44.779 region of this squamous cell carcinoma here.
NOTE Confidence: 0.7735238
00:32:44.780 --> 00:32:46.705 6 expressed almost across the
NOTE Confidence: 0.7735238
00:32:46.705 --> 00:32:49.071 entire tissue and then here is
NOTE Confidence: 0.7735238
00:32:49.071 --> 00:32:50.926 an interesting example of MDM.
NOTE Confidence: 0.7735238
00:32:50.926 --> 00:32:53.228 To be expressed in the cytoplasm,
NOTE Confidence: 0.7735238
00:32:53.228 --> 00:32:54.355 not the nucleus.
NOTE Confidence: 0.7735238
00:32:54.355 --> 00:32:56.230 So we really don’t understand
NOTE Confidence: 0.7735238
00:32:56.230 --> 00:32:58.589 what it’s doing in the cytoplasm,
NOTE Confidence: 0.7735238
00:32:58.590 --> 00:33:02.033 but not in all three of these experiments, P.
NOTE Confidence: 0.7735238
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
00:33:50.613 --> 00:33:53.817 actually doing in with regards to transformation and tumor evolution.

00:33:53.817 --> 00:33:55.979 So here’s what happens.

00:33:55.980 --> 00:34:01.501 So this is a normal control and the left these are mouse cells express a normal number of mouse chroma zones,

00:34:01.501 --> 00:34:04.665 and when we overexpressed MDM two we see this incredibly abnormal.

00:34:04.665 --> 00:34:07.790 Chromosome instability we can quantify the numbers of fusions here and we have a huge number of fusions.

00:34:07.790 --> 00:34:11.042 We also have a lot of fragments.

00:34:11.042 --> 00:34:14.180 So in data from multiple labs, if you overexpress MDM two in a normal cell the cell just dies.
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

NOTE Confidence: 0.8437264
mutations in P53IN animal models and
we show that these mice are tumor pro.
But more importantly in contrast.
These mice have a high metastatic capability,
so this here is our data from the
172 mutation corresponds to the
mutation, which is one of the hot spot
175 mutation, and here you can see a metastasis
to the liver, and here stained with the P53 antibody,
and here stained with the P53 antibody,
a metastasis to the brain.
And this is in contrast to mice
that have deletions of 353,
00:36:39.870 --> 00:36:42.705 so this really was the first example

00:36:42.705 --> 00:36:44.384 that suggested that expressing

00:36:44.384 --> 00:36:46.736 a mutant P53 was much more.

00:36:48.880 --> 00:36:50.872 Much more aggressive than not having

00:36:50.872 --> 00:36:53.259 people to create and and in the field.

00:36:53.260 --> 00:36:55.444 We call this a gain of function.

00:36:55.450 --> 00:36:57.962 Mutant P 53 is doing something in these

00:36:57.962 --> 00:37:00.500 cells to make them highly metastatic.

00:37:00.500 --> 00:37:03.636 So these are germline mice and what we

00:37:03.636 --> 00:37:10.018 wanted to do is to generate semantic

00:37:06.966 --> 00:37:13.623 models because the these germline models

00:37:13.623 --> 00:37:16.480 represent Lee from Many syndrome which is

00:37:16.480 --> 00:37:20.324 an inheritance of people to mutations.

00:37:20.324 --> 00:37:22.894 But that’s a rare syndrome and we really

00:37:22.894 --> 00:37:26.190 wanted to understand this metastatic

NOTE Confidence: 0.8372316
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 it’s wild type because we earned started seeding a sequence upstream of the point mutation and this 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.

So this is just showing me wild type initially and then we committed fashion.
00:39:22.630 --> 00:39:25.134 You make them into protein and you can
make it in akhri dependent manner.

00:39:25.134 --> 00:39:27.677 So this just shows you how normal
those mice are.

00:39:30.403 --> 00:39:31.570 So here we're comparing wild type 2
heterozygous mice with the 172 or the 2.5.

00:39:34.608 --> 00:39:37.407 We stabilized the mutant protein
in response to DNA damage.

00:39:37.410 --> 00:39:39.355 And when we look at the activation
of the three targets be 21 in Puma,

00:39:39.355 --> 00:39:41.300 they were activated to similar levels.

00:39:41.300 --> 00:39:44.037 No difference between these
two alleles in wild type mice.

00:39:44.037 --> 00:39:47.128 Of DNA damage to induce labor ptosis?

00:39:47.130 --> 00:39:51.108 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.

And this is the last experiment I’ll show you about the the actual alleles. 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 bout 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.
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.

So here is the.

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.

And then here on the right you can see.

A high titer virus was used was injected into this gland in about 50 to 70% of the ductal cells are checked.
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 belsy 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...
00:43:30.005 --> 00:43:32.660 that contributes to tumor phenotype.

00:43:32.660 --> 00:43:36.980 So with a low titer we begin to see tumors.

00:43:36.980 --> 00:43:40.004 We sell one at a higher titer.

00:43:40.010 --> 00:43:43.082 We now solve 4 tumors and

00:43:43.082 --> 00:43:44.618 one tumor metastasized.

00:43:44.620 --> 00:43:47.455 The 2.5 allele was a much stronger,

00:43:47.460 --> 00:43:49.480 had a much stronger tumor,

00:43:49.480 --> 00:43:51.100 phenotype with low titer.

00:43:51.100 --> 00:43:54.799 We saw four tumors and one of them was

00:43:54.799 --> 00:43:57.578 meta static with the high tier tighter.

00:43:57.580 --> 00:44:00.820 We saw nine tumors, so this is about

00:44:00.820 --> 00:44:04.058 75% and more than half were meta static.

00:44:04.060 --> 00:44:07.300 So let me kind of summarize all the data

00:44:07.300 --> 00:44:10.140 that we’ve done with these animals.

00:44:10.140 --> 00:44:12.564 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 just wanted to figure out what would happen with the minimal number of alterations.

So if you compare the ones need two hitters 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 28. 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. We saw mostly luminal B and then here with the 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.
sample with the 2:45 we see off the tumor, molecular subtypes evolving and so one of the experiments that we’re doing now is trying to understand with this 245 mutation, what are the triggers to these different subtypes? Triple negative breast cancer is very hard to treat. But for example, here to enrich tumors you can, you can treat with her two antibodies, so we’re trying to understand basically the tumor evolution that initiates with this one, P. 53 missense mutation.
We’ve also wanted to see 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.
00:47:42.929 --> 00:47:45.652 so the one of the last experiments
00:47:45.652 --> 00:47:49.489 I'll show you here is just trying to
00:47:49.489 --> 00:47:51.469 understand tumor evolution because
00:47:51.553 --> 00:47:54.277 we made a semantic model that
00:47:54.277 --> 00:47:56.551 develop different kinds of breast
00:47:56.551 --> 00:47:58.806 cancers that were highly metastatic.
00:47:58.810 --> 00:48:01.378 And so I'm really interested in
00:48:01.378 --> 00:48:04.591 understanding the task sees in an in
00:48:04.591 --> 00:48:06.495 vivo physiologically relevant system.
00:48:06.500 --> 00:48:07.826 So we did.
00:48:07.826 --> 00:48:10.920 Here is we took the 2:45 mutant
00:48:11.032 --> 00:48:13.140 animals and we took.
00:48:13.140 --> 00:48:15.108 UH-22 memory tumors from these mice
00:48:15.108 --> 00:48:17.224 we sequenced them in three different
00:48:17.224 --> 00:48:19.069 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, 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, there’s only one late mutation common. So with these sequencing data, indicate is that these matasa left the tumor very early.
during the metastatic process and then seated and had additional changes. So this was the first suggestion that maybe metastasis.

Breast cancer metastasis driven by a new P53 is an early event. So to summarize, this model just briefly, we can make.

P 3 point mutation in just a few cells that become a tumor that migrate, proliferate and develop these metastases.

Where we now have I called it a little factory but we just have these mice now developing tumors. 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
00:51:05.693 --> 00:51:06.956 So again this.
NOTE Confidence: 0.7901794
00:51:06.960 --> 00:51:10.061 This suggests that you know South normal cell tumor cell interactions are altering the kinds of tumors that come up.
NOTE Confidence: 0.7901794
00:51:10.061 --> 00:51:12.877 And in this scenario we also had a cast 9 allele that is Creed dependent,
NOTE Confidence: 0.7901794
00:51:12.877 --> 00:51:16.146 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
00:51:22.720 --> 00:51:26.131 cast 9 allele that is Creed dependent,
NOTE Confidence: 0.7901794
00:51:22.720 --> 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
00:51:26.131 --> 00:51:29.270 And in this scenario we also had a cast 9 allele that is Creed dependent,
NOTE Confidence: 0.7901794
00:51:29.270 --> 00:51:32.083 and to express castanon so we can use CRISPR technologies to to begin to address vulnerabilities.
NOTE Confidence: 0.7901794
00:51:32.083 --> 00:51:35.380 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
00:51:35.380 --> 00:51:38.615 And in this scenario we also had a cast 9 allele that is Creed dependent,
NOTE Confidence: 0.7901794
00:51:38.615 --> 00:51:40.819 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 53, 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 53, 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

NOTE Confidence: 0.7901794

NOTE Confidence: 0.7901794

NOTE Confidence: 0.7901794

NOTE Confidence: 0.7901794

NOTE Confidence: 0.7901794

NOTE Confidence: 0.8528144

NOTE Confidence: 0.8528144

NOTE Confidence: 0.8528144

NOTE Confidence: 0.8528144

NOTE Confidence: 0.8528144

NOTE Confidence: 0.8528144

NOTE Confidence: 0.8528144

NOTE Confidence: 0.8528144

NOTE Confidence: 0.8528144

NOTE Confidence: 0.8528144
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 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.
00:54:22.164 --> 00:54:25.089 for us 'cause there's no way I can

00:54:25.089 --> 00:54:27.480 delete every one of these targets

00:54:27.480 --> 00:54:30.357 and see how important they are in

00:54:30.357 --> 00:54:32.978 vivo and then last but not least,

00:54:32.980 --> 00:54:35.500 I showed you the generation of of

00:54:35.500 --> 00:54:37.737 this novel mouse model that really

00:54:37.737 --> 00:54:40.257 allows us now to make a semantic

00:54:40.334 --> 00:54:41.918 point potential 53 in.

00:54:41.920 --> 00:54:45.000 Any cell of origin that we want

00:54:45.000 --> 00:54:48.637 to an in in the breast model.

00:54:48.640 --> 00:54:49.139 Specifically,

00:54:49.139 --> 00:54:52.632 we had a highly metastatic phenotype that

00:54:52.632 --> 00:54:55.839 we're trying to understand in more detail.

00:54:55.840 --> 00:54:56.291 OK,

00:54:56.291 --> 00:54:59.448 so my last slide is just the

NOTE Confidence: 0.8528144

82
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.
We're really wondering what the mechanisms is acquire these tumors imploding when they no longer have communities industry.

So with that, I'll end in, oh, I'm glad to answer any questions. Thank you so much. That was a wonderful talk.

I'm going to ask people to put questions in the chat, but I wanted to ask you, sort of, from the therapeutics perspective, people have been very interested in compounds like Prima and Cody that assist with re folding of P53 with
disruptive mutation and yet clinically those have been a little bit disappointing. Is it 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.

I think that our. Our. That that 7 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, yeah, I think having a common set of.

I mean I think people have just looked at P 21 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, and in DNA sequencing to understand both what happens if Arnie level.
I think that 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 Protex 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. Taking it out of the picture to see what happens.
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 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.