WEBVTT

NOTE duration:"00:56:17"

NOTE recognizability:0.873

NOTE language:en-us

NOTE Confidence: 0.8659279425

00:00:00.000 --> 00:00:02.464 Represent some of the work that my lab

NOTE Confidence: 0.8659279425

00:00:02.464 --> 00:00:05.360 has been up to in the last few years.

NOTE Confidence: 0.8659279425

00:00:05.360 --> 00:00:08.280 And as you will see it's it's quite

NOTE Confidence: 0.8659279425

00:00:08.280 --> 00:00:09.665 different and perhaps different from

NOTE Confidence: 0.8659279425

00:00:09.665 --> 00:00:11.600 what you expect from our prior work.

NOTE Confidence: 0.8659279425

00:00:11.600 --> 00:00:13.598 But it's sort of the next wave that I,

NOTE Confidence: 0.8659279425

00:00:13.600 --> 00:00:16.435 I, I sort of view important in

NOTE Confidence: 0.8659279425

00:00:16.440 --> 00:00:18.274 building on what we have done before.

NOTE Confidence: 0.8659279425

00:00:18.280 --> 00:00:20.583 So today I will talk about really

NOTE Confidence: 0.8659279425

00:00:20.583 --> 00:00:22.435 different shades of cancer immunivation

NOTE Confidence: 0.8659279425

00:00:22.435 --> 00:00:25.221 as you'll see and some strategies that

NOTE Confidence: 0.8659279425

00:00:25.221 --> 00:00:27.422 we're developing to overcome these

NOTE Confidence: 0.8659279425

00:00:27.422 --> 00:00:29.597 from multiple at different angles.

NOTE Confidence: 0.8659279425

00:00:29.600 --> 00:00:30.576 So just very briefly,

NOTE Confidence: 0.8659279425

00:00:30.576 --> 00:00:32.919 you know if I had to summarize sort of

NOTE Confidence: 0.8659279425

00:00:32.919 --> 00:00:35.240 what our lab is doing at this point,

NOTE Confidence: 0.8659279425

00:00:35.240 --> 00:00:37.238 it's really you know doing large

NOTE Confidence: 0.8659279425

00:00:37.238 --> 00:00:38.570 scale genomics to understand

NOTE Confidence: 0.8659279425

00:00:38.625 --> 00:00:40.370 cancer genetics using single cell

NOTE Confidence: 0.8659279425

00:00:40.370 --> 00:00:42.115 genomics and things like that.

NOTE Confidence: 0.8659279425

00:00:42.120 --> 00:00:43.595 But that's really the beginning

NOTE Confidence: 0.8659279425

00:00:43.595 --> 00:00:45.479 really want to use this information

NOTE Confidence: 0.8659279425

00:00:45.479 --> 00:00:47.224 to inform you know mechanisms

NOTE Confidence: 0.8659279425

00:00:47.224 --> 00:00:49.000 that underlie two major areas.

NOTE Confidence: 0.8659279425

00:00:49.000 --> 00:00:52.143 One is immunobiology and the other one

NOTE Confidence: 0.8659279425

00:00:52.143 --> 00:00:54.296 is metastatic organotropism which is

NOTE Confidence: 0.8659279425

00:00:54.296 --> 00:00:56.624 really critical to consider when we use

NOTE Confidence: 0.8659279425

00:00:56.624 --> 00:00:59.599 an immune base or or even other therapies.

NOTE Confidence: 0.8659279425

00:00:59.600 --> 00:01:00.200 The approach,

NOTE Confidence: 0.8659279425

00:01:00.200 --> 00:01:03.046 as you will see in a minute also is

NOTE Confidence: 0.8659279425

00:01:03.046 --> 00:01:05.278 typically so that our questions are

NOTE Confidence: 0.8659279425

00:01:05.278 --> 00:01:07.270 really inspired by clinical problems

NOTE Confidence: 0.8659279425

00:01:07.270 --> 00:01:09.619 and then broken down into models.

NOTE Confidence: 0.8659279425

00:01:09.619 --> 00:01:11.314 Frequently you have to develop

NOTE Confidence: 0.8659279425

00:01:11.314 --> 00:01:13.304 those models or methods to study

NOTE Confidence: 0.8659279425

00:01:13.304 --> 00:01:14.272 these things systematically.

NOTE Confidence: 0.8659279425

00:01:14.272 --> 00:01:15.056 And ultimately,

NOTE Confidence: 0.8659279425

00:01:15.056 --> 00:01:15.840 of course,

NOTE Confidence: 0.8659279425

00:01:15.840 --> 00:01:18.620 the goal is to bring this back to patients.

NOTE Confidence: 0.8659279425

00:01:18.620 --> 00:01:22.540 So I divided the talk into three chapters

NOTE Confidence: 0.8659279425

00:01:22.634 --> 00:01:25.120 that are loosely linked to each other,

NOTE Confidence: 0.8659279425

00:01:25.120 --> 00:01:26.998 as you'll see in a moment.

NOTE Confidence: 0.8659279425

00:01:27.000 --> 00:01:29.472 And I want to start with the first

NOTE Confidence: 0.8659279425

00:01:29.472 --> 00:01:31.726 one because I think it really

NOTE Confidence: 0.8659279425

00:01:31.726 --> 00:01:34.220 exemplifies how we can use information

NOTE Confidence: 0.8659279425

00:01:34.220 --> 00:01:36.892 from large genomic data to inform

NOTE Confidence: 0.8659279425

00:01:36.892 --> 00:01:39.312 precisely what mechanisms and sort

NOTE Confidence: 0.8659279425

00:01:39.312 --> 00:01:42.678 of things we should study in the lab.

NOTE Confidence: 0.8659279425

00:01:42.680 --> 00:01:45.008 So a few years ago we were really

NOTE Confidence: 0.8659279425

00:01:45.008 --> 00:01:46.143 interested in understanding

NOTE Confidence: 0.8659279425

00:01:46.143 --> 00:01:48.563 mechanisms of resistance to immune

NOTE Confidence: 0.8659279425

00:01:48.563 --> 00:01:50.898 checkpoint inhibitors and you know

NOTE Confidence: 0.8659279425

00:01:50.898 --> 00:01:52.914 the details don't matter so much,

NOTE Confidence: 0.8659279425

00:01:52.920 --> 00:01:55.288 but the approach was that we use single

NOTE Confidence: 0.8659279425

00:01:55.288 --> 00:01:57.523 cell RNA sequencing in these patients.

NOTE Confidence: 0.8659279425

00:01:57.523 --> 00:01:59.770 We looked at the cancer cells specifically

NOTE Confidence: 0.8659279425

00:01:59.824 --> 00:02:01.888 and we came up with this you know

NOTE Confidence: 0.8659279425

00:02:01.888 --> 00:02:03.720 signature which we called ICR signature,

NOTE Confidence: 0.8659279425

00:02:03.720 --> 00:02:05.312 immune checkpoint resistant signature

NOTE Confidence: 0.8659279425

00:02:05.312 --> 00:02:08.036 and again the the genes don't matter

NOTE Confidence: 0.8659279425

00:02:08.036 --> 00:02:09.980 so much but you know and and I think

NOTE Confidence: 0.8659279425

00:02:09.980 --> 00:02:11.650 we learned a lot in this study but

NOTE Confidence: 0.8659279425

00:02:11.650 --> 00:02:13.408 we're what we were left with is a

NOTE Confidence: 0.8659279425

00:02:13.408 --> 00:02:14.906 hypothesis that came out of the data

NOTE Confidence: 0.8659279425

00:02:14.906 --> 00:02:18.198 that was that this cancer cell intrinsic

NOTE Confidence: 0.8659279425

00:02:18.198 --> 00:02:21.223 program was somehow conferring T cell

NOTE Confidence: 0.8659279425

00:02:21.223 --> 00:02:23.881 exclusion or poor T cell infiltration

NOTE Confidence: 0.8659279425

00:02:23.881 --> 00:02:25.616 and impaired T cell activity.

NOTE Confidence: 0.8659279425

00:02:25.616 --> 00:02:27.688 But when you have these gene lists

NOTE Confidence: 0.8659279425

00:02:27.688 --> 00:02:29.997 which I know all of you have been at,

NOTE Confidence: 0.8659279425

00:02:30.000 --> 00:02:31.836 at one point in your project,

NOTE Confidence: 0.8659279425

00:02:31.840 --> 00:02:34.136 the question is how do you actually

NOTE Confidence: 0.8659279425

00:02:34.136 --> 00:02:35.679 prioritize what you're going to

NOTE Confidence: 0.8659279425

00:02:35.679 --> 00:02:37.436 look at right and and what not.

NOTE Confidence: 0.8659279425

00:02:37.440 --> 00:02:39.064 And typically what we do and this

NOTE Confidence: 0.8659279425

00:02:39.064 --> 00:02:41.079 is what we had done for this paper,

NOTE Confidence: 0.8659279425

00:02:41.080 --> 00:02:43.330 we focus on something that's plausible

NOTE Confidence: 0.8659279425

00:02:43.330 --> 00:02:46.263 for which there are reagents or other

NOTE Confidence: 0.8659279425

00:02:46.263 --> 00:02:48.075 practical or pragmatic reasons.

NOTE Confidence: 0.8659279425

00:02:48.080 --> 00:02:50.246 But we really wanted to make

NOTE Confidence: 0.8659279425

00:02:50.246 --> 00:02:51.690 this process of how

NOTE Confidence: 0.934265072352941

00:02:51.775 --> 00:02:54.638 we validate things a bit more unbiased

NOTE Confidence: 0.934265072352941

00:02:54.640 --> 00:02:57.544 and to do that we developed this method

NOTE Confidence: 0.934265072352941

00:02:57.544 --> 00:03:00.240 which we dubbed Perturb site seek.

NOTE Confidence: 0.934265072352941

00:03:00.240 --> 00:03:02.292 So basically what this method allows

NOTE Confidence: 0.934265072352941

00:03:02.292 --> 00:03:05.580 you to do is couple CRISPR CAS 9

NOTE Confidence: 0.934265072352941

00:03:05.580 --> 00:03:07.840 perturbations with single cell RNA

NOTE Confidence: 0.934265072352941

00:03:07.923 --> 00:03:10.251 and protein profile using the site

NOTE Confidence: 0.934265072352941

00:03:10.251 --> 00:03:13.319 seek and a 10X genomics platform.

NOTE Confidence: 0.934265072352941

00:03:13.320 --> 00:03:15.720 So what you would get from this method

NOTE Confidence: 0.934265072352941

00:03:15.720 --> 00:03:17.887 would be that you could perturb a

NOTE Confidence: 0.934265072352941

00:03:17.887 --> 00:03:20.468 gene and then ask well what does it

NOTE Confidence: 0.934265072352941

00:03:20.468 --> 00:03:22.496 do to the entire cells transcriptome

NOTE Confidence: 0.934265072352941

00:03:22.496 --> 00:03:24.960 and part of the surface proteome.

NOTE Confidence: 0.934265072352941

00:03:24.960 --> 00:03:27.536 And the way we use this method to

NOTE Confidence: 0.934265072352941

00:03:27.536 --> 00:03:29.597 validate some of those findings that

NOTE Confidence: 0.934265072352941

00:03:29.597 --> 00:03:31.594 we had in patients is leveraging

NOTE Confidence: 0.934265072352941

00:03:31.594 --> 00:03:33.792 it in in in patient derived models

NOTE Confidence: 0.934265072352941

00:03:33.792 --> 00:03:35.856 where we had isolated in addition

NOTE Confidence: 0.934265072352941

00:03:35.856 --> 00:03:37.914 to doing the sequencing the cancer

NOTE Confidence: 0.934265072352941

00:03:37.980 --> 00:03:40.392 cells from a Melanoma patient and

NOTE Confidence: 0.934265072352941

00:03:40.392 --> 00:03:42.000 the tumor infiltrating lymphocytes.

NOTE Confidence: 0.934265072352941

00:03:42.000 --> 00:03:43.442 The advantage is that this is a

NOTE Confidence: 0.934265072352941

00:03:43.442 --> 00:03:44.919 you know fully autologous system.

NOTE Confidence: 0.934265072352941

00:03:44.920 --> 00:03:47.110 It doesn't require any sort of

NOTE Confidence: 0.934265072352941

00:03:47.110 --> 00:03:49.661 engineering if you will to do a

NOTE Confidence: 0.934265072352941

00:03:49.661 --> 00:03:51.600 Co culture experiments into these

NOTE Confidence: 0.934265072352941

00:03:51.600 --> 00:03:53.440 cancer cells we generated.

NOTE Confidence: 0.934265072352941

00:03:53.440 --> 00:03:55.348 We generated a library that would

NOTE Confidence: 0.934265072352941

00:03:55.348 --> 00:03:58.107 target each of the genes that we had

NOTE Confidence: 0.934265072352941

00:03:58.107 --> 00:04:00.237 identified in the signature in patients.

NOTE Confidence: 0.934265072352941

00:04:00.240 --> 00:04:03.300 So we could test in one you know pooled

NOTE Confidence: 0.934265072352941

00:04:03.300 --> 00:04:05.639 experiment the relevance of any one of

NOTE Confidence: 0.934265072352941

00:04:05.639 --> 00:04:07.878 those 248 genes that were in the signature.

NOTE Confidence: 0.934265072352941

00:04:07.880 --> 00:04:09.872 And not only that but also we would

NOTE Confidence: 0.934265072352941

00:04:09.872 --> 00:04:11.471 get you know the transcriptome

NOTE Confidence: 0.934265072352941

00:04:11.471 --> 00:04:13.553 and part of the surface podium.

NOTE Confidence: 0.934265072352941

00:04:13.560 --> 00:04:16.248 And the premise here would be that

NOTE Confidence: 0.934265072352941

00:04:16.248 --> 00:04:18.559 cells that survive Co culture with

NOTE Confidence: 0.934265072352941

00:04:18.560 --> 00:04:22.140 their autologous T cells must harbour a

NOTE Confidence: 0.934265072352941

00:04:22.140 --> 00:04:24.240 perturbation that confers that mechanism.

NOTE Confidence: 0.934265072352941

00:04:24.240 --> 00:04:26.676 And this is exactly what we did.

NOTE Confidence: 0.934265072352941

00:04:26.680 --> 00:04:28.368 And I'm just going to give you this

NOTE Confidence: 0.934265072352941

00:04:28.368 --> 00:04:30.204 is of course published at this point.

NOTE Confidence: 0.934265072352941

00:04:30.204 --> 00:04:32.246 But what what this would get us is

NOTE Confidence: 0.934265072352941

00:04:32.246 --> 00:04:33.730 a sense of the immune fitness and

NOTE Confidence: 0.934265072352941

00:04:33.730 --> 00:04:35.520 the phenotype associated with this.

NOTE Confidence: 0.934265072352941

00:04:35.520 --> 00:04:37.672 I'm just going to give a snippet of

NOTE Confidence: 0.934265072352941

00:04:37.672 --> 00:04:39.661 results because I want to show you how

NOTE Confidence: 0.934265072352941

00:04:39.661 --> 00:04:41.555 this helped us inform kind of what we

NOTE Confidence: 0.934265072352941

00:04:41.555 --> 00:04:43.868 did next in the last couple of years.

NOTE Confidence: 0.934265072352941

00:04:43.868 --> 00:04:46.552 So here we're looking at gene

NOTE Confidence: 0.934265072352941

00:04:46.552 --> 00:04:47.800 knockouts on the bottom.

NOTE Confidence: 0.934265072352941

00:04:47.800 --> 00:04:50.824 So genes knocked out here associated

NOTE Confidence: 0.934265072352941

00:04:50.824 --> 00:04:52.840 with increasing fitness against

NOTE Confidence: 0.934265072352941

00:04:52.922 --> 00:04:54.997 autologous T cells or tilts.

NOTE Confidence: 0.934265072352941

00:04:55.000 --> 00:04:57.212 And on the Y axis here we

NOTE Confidence: 0.934265072352941

00:04:57.212 --> 00:04:58.160 increased immune pressure.

NOTE Confidence: 0.934265072352941

00:04:58.160 --> 00:05:00.644 So there's an effector to target ratio of 1

NOTE Confidence: 0.934265072352941

00:05:00.644 --> 00:05:03.320 to 1 to the one and four to one and so on.

NOTE Confidence: 0.934265072352941

00:05:03.320 --> 00:05:04.520 And as you would expect,

NOTE Confidence: 0.934265072352941

00:05:04.520 --> 00:05:06.480 mutations or deletions in all of the

NOTE Confidence: 0.934265072352941

00:05:06.480 --> 00:05:08.917 genes that I indicate here with the red,

NOTE Confidence: 0.934265072352941

00:05:08.920 --> 00:05:09.640 Red Arrows,

NOTE Confidence: 0.934265072352941

00:05:09.640 --> 00:05:12.160 all of those had been known before,

NOTE Confidence: 0.934265072352941

00:05:12.160 --> 00:05:12.559 right.

NOTE Confidence: 0.934265072352941

00:05:12.559 --> 00:05:14.554 Mutations or deletions in those

NOTE Confidence: 0.934265072352941

00:05:14.554 --> 00:05:16.150 genes are strongly associated

NOTE Confidence: 0.934265072352941

00:05:16.215 --> 00:05:18.471 with immune evasion and they had

NOTE Confidence: 0.934265072352941

00:05:18.471 --> 00:05:19.975 been associated clinically with

NOTE Confidence: 0.934265072352941

00:05:20.035 --> 00:05:21.679 resistance to immunotherapy.

NOTE Confidence: 0.934265072352941

00:05:21.680 --> 00:05:23.270 So that was really good because

NOTE Confidence: 0.934265072352941

00:05:23.270 --> 00:05:24.843 it validated our approach that we

NOTE Confidence: 0.934265072352941

00:05:24.843 --> 00:05:26.235 could recover all of those hits.

NOTE Confidence: 0.934265072352941

00:05:26.240 --> 00:05:27.552 Basically in one experiment,

NOTE Confidence: 0.934265072352941

00:05:27.552 --> 00:05:29.520 we got really interested in another

NOTE Confidence: 0.934265072352941

00:05:29.575 --> 00:05:30.960 hit that was less expected.

NOTE Confidence: 0.934265072352941

00:05:30.960 --> 00:05:33.552 That was loss of a gene called CD 58.

NOTE Confidence: 0.934265072352941

00:05:33.560 --> 00:05:34.160 So I'm going to get,

NOTE Confidence: 0.934265072352941

00:05:34.160 --> 00:05:35.756 I'm going to talk about that more.

NOTE Confidence: 0.934265072352941

00:05:35.760 --> 00:05:36.720 But on the flip side,

NOTE Confidence: 0.934265072352941

00:05:36.720 --> 00:05:38.808 we could also couple how these

NOTE Confidence: 0.934265072352941

00:05:38.808 --> 00:05:40.200 perturbations change the phenotype.

NOTE Confidence: 0.938170043

00:05:40.200 --> 00:05:41.664 And as you can imagine this

NOTE Confidence: 0.938170043

00:05:41.664 --> 00:05:43.330 is a huge matrix, you know,

NOTE Confidence: 0.938170043

00:05:43.330 --> 00:05:45.400 perturbation by gene expression by by,

NOTE Confidence: 0.938170043

00:05:45.400 --> 00:05:46.400 you know, protein profile.

NOTE Confidence: 0.938170043

00:05:46.400 --> 00:05:48.479 So I'm just going to show you a tiny,

NOTE Confidence: 0.938170043

00:05:48.480 --> 00:05:50.664 tiny snippet from that which I

NOTE Confidence: 0.938170043

00:05:50.664 --> 00:05:53.096 want to use to guide you to the

NOTE Confidence: 0.938170043

00:05:53.096 --> 00:05:54.560 to the experiments that we did.

NOTE Confidence: 0.938170043

00:05:54.560 --> 00:05:56.432 So the way to read this here is that

NOTE Confidence: 0.938170043

00:05:56.432 --> 00:05:58.112 on the bottom you have knockouts

NOTE Confidence: 0.938170043

00:05:58.112 --> 00:06:00.072 of those genes and then on the

NOTE Confidence: 0.938170043

00:06:00.072 --> 00:06:01.787 Y axis you have a few selected

NOTE Confidence: 0.938170043

00:06:01.787 --> 00:06:04.044 features that I I want to present.

NOTE Confidence: 0.938170043

00:06:04.044 --> 00:06:07.276 So for example, if you knockout CD 58,

NOTE Confidence: 0.938170043

00:06:07.280 --> 00:06:09.282 then there will be no CD 58

NOTE Confidence: 0.938170043

00:06:09.282 --> 00:06:10.879 protein left in that cell.

NOTE Confidence: 0.938170043

00:06:10.880 --> 00:06:11.323 Logical.

NOTE Confidence: 0.938170043

00:06:11.323 --> 00:06:14.424 But what was really interesting was the

NOTE Confidence: 0.938170043

00:06:14.424 --> 00:06:17.160 observation that cells that lose this gene,

NOTE Confidence: 0.938170043

00:06:17.160 --> 00:06:21.040 CD 58 had concurrently more

NOTE Confidence: 0.938170043

00:06:21.040 --> 00:06:24.280 protein by of a gene encoded

NOTE Confidence: 0.938170043

00:06:24.280 --> 00:06:26.750 by a protein encoded by CD 274,

NOTE Confidence: 0.938170043

00:06:26.750 --> 00:06:28.160 which of course is PDL 1.

NOTE Confidence: 0.938170043

00:06:28.160 --> 00:06:29.756 So that seemed like a double whammy.

NOTE Confidence: 0.938170043

00:06:29.760 --> 00:06:32.210 You lose something good that

NOTE Confidence: 0.938170043

00:06:32.210 --> 00:06:33.680 confers immune evasion,

NOTE Confidence: 0.938170043

00:06:33.680 --> 00:06:35.430 but then you also gain something that

NOTE Confidence: 0.938170043

00:06:35.430 --> 00:06:37.797 is a Co inhibitory ligand of course.

NOTE Confidence: 0.938170043

00:06:37.800 --> 00:06:39.200 So what is CD58?

NOTE Confidence: 0.938170043

00:06:39.200 --> 00:06:41.300 It turns out we actually don't

NOTE Confidence: 0.938170043

00:06:41.382 --> 00:06:43.916 know that much about it in cancer.

NOTE Confidence: 0.938170043

00:06:43.920 --> 00:06:45.900 What we do know physiologically is

NOTE Confidence: 0.938170043

00:06:45.900 --> 00:06:48.320 that it is a Co stimulatory protein

NOTE Confidence: 0.938170043

00:06:48.320 --> 00:06:51.400 that ligates to CD2 on T cells,

NOTE Confidence: 0.938170043

00:06:51.400 --> 00:06:52.280 and when it does so,

NOTE Confidence: 0.938170043

00:06:52.280 --> 00:06:54.848 it can become actually the most

NOTE Confidence: 0.938170043

00:06:54.848 --> 00:06:56.560 potent Co stimulatory protein.

NOTE Confidence: 0.938170043

00:06:56.560 --> 00:06:58.996 So loss of this gene of protein,

NOTE Confidence: 0.938170043

00:06:59.000 --> 00:07:01.040 it's plausible that that could

NOTE Confidence: 0.938170043

00:07:01.040 --> 00:07:02.420 result in immune evasion in

NOTE Confidence: 0.938170043

00:07:02.420 --> 00:07:03.800 a number of different ways.

NOTE Confidence: 0.938170043

00:07:03.800 --> 00:07:05.660 So we sought to validate this

NOTE Confidence: 0.938170043

00:07:05.660 --> 00:07:07.986 and this is work that has since

NOTE Confidence: 0.938170043

00:07:07.986 --> 00:07:10.008 then been LED and then published

NOTE Confidence: 0.938170043

00:07:10.008 --> 00:07:11.944 by my first MDPHD student who

NOTE Confidence: 0.938170043

00:07:11.944 --> 00:07:13.912 just graduated a few months ago.

NOTE Confidence: 0.938170043

00:07:13.920 --> 00:07:15.705 So here basically what she did is

NOTE Confidence: 0.938170043

00:07:15.705 --> 00:07:17.319 she took these Melanoma cells,

NOTE Confidence: 0.938170043

00:07:17.320 --> 00:07:19.624 she knocked out CD58 and then she Co

NOTE Confidence: 0.938170043

00:07:19.624 --> 00:07:21.540 cultured the cells with autologous T

NOTE Confidence: 0.938170043

00:07:21.540 --> 00:07:24.159 cells or engineered T cells at that point.

NOTE Confidence: 0.938170043

00:07:24.160 --> 00:07:25.744 And as you can see loss of the

NOTE Confidence: 0.938170043

00:07:25.744 --> 00:07:27.423 gene in fact convert a better

NOTE Confidence: 0.938170043

00:07:27.423 --> 00:07:28.958 survival of these cancer cells.

NOTE Confidence: 0.938170043

00:07:28.960 --> 00:07:31.040 And when you rescue these,

NOTE Confidence: 0.938170043

00:07:31.040 --> 00:07:33.950 the gene, either it's GPI anchored

NOTE Confidence: 0.938170043

00:07:33.950 --> 00:07:35.384 or transmembrane isoform,

NOTE Confidence: 0.938170043

00:07:35.384 --> 00:07:37.704 then you rescue the sensitivity

NOTE Confidence: 0.938170043

00:07:37.704 --> 00:07:39.560 to T cell cleaning.

NOTE Confidence: 0.938170043

00:07:39.560 --> 00:07:40.032 Furthermore,

NOTE Confidence: 0.938170043

00:07:40.032 --> 00:07:42.392 we also wanted to demonstrate

NOTE Confidence: 0.938170043

00:07:42.392 --> 00:07:44.744 that this interaction with CD2

NOTE Confidence: 0.938170043

00:07:44.744 --> 00:07:47.120 was in fact required for this

NOTE Confidence: 0.938170043

00:07:47.120 --> 00:07:49.000 immune evasion phenotype and that,

NOTE Confidence: 0.938170043

00:07:49.000 --> 00:07:49.880 you know,

NOTE Confidence: 0.938170043

00:07:49.880 --> 00:07:52.704 CD 58 loss didn't confer a loss

NOTE Confidence: 0.938170043

00:07:52.704 --> 00:07:54.264 of fitness through some other

NOTE Confidence: 0.938170043

00:07:54.264 --> 00:07:55.959 mechanism that we didn't know.

NOTE Confidence: 0.938170043

00:07:55.960 --> 00:07:58.264 So to test this we repeated the same

NOTE Confidence: 0.938170043

00:07:58.264 --> 00:07:59.959 experiment that I show on the left,

NOTE Confidence: 0.938170043

00:07:59.960 --> 00:08:02.228 only this time we rescued the

NOTE Confidence: 0.938170043

00:08:02.228 --> 00:08:04.560 knockout cells with a variant of

NOTE Confidence: 0.938170043

00:08:04.560 --> 00:08:07.713 CD58 harboring and mutation K34A

NOTE Confidence: 0.938170043

00:08:07.713 --> 00:08:09.952 which is unable to actually to CD2.

NOTE Confidence: 0.938170043

00:08:09.952 --> 00:08:11.840 And as you can see when you rest,

NOTE Confidence: 0.938170043

00:08:11.840 --> 00:08:13.358 when we rescue with the mutant,

NOTE Confidence: 0.938170043

00:08:13.360 --> 00:08:15.190 the cells continue to be resistant

NOTE Confidence: 0.938170043

00:08:15.190 --> 00:08:17.728 to D cell Co culture suggesting that

NOTE Confidence: 0.938170043

00:08:17.728 --> 00:08:21.440 this is very specific to that interaction.

NOTE Confidence: 0.938170043

00:08:21.440 --> 00:08:23.472 So one of the reasons I think this

NOTE Confidence: 0.938170043

00:08:23.472 --> 00:08:25.590 gene is not well understood is that

NOTE Confidence: 0.938170043

00:08:25.590 --> 00:08:27.680 there is no known mouse homolog.

NOTE Confidence: 0.938170043

00:08:27.680 --> 00:08:30.488 So we can't use the, you know,

NOTE Confidence: 0.938170043

00:08:30.488 --> 00:08:32.760 models that we typically like to use in,

NOTE Confidence: 0.799237034

00:08:32.760 --> 00:08:34.280 you know, studying, you know,

NOTE Confidence: 0.799237034

00:08:34.280 --> 00:08:36.800 immunotherapy and so on syngenic model.

NOTE Confidence: 0.799237034

00:08:36.800 --> 00:08:38.508 So to to you know, circumvent this,

NOTE Confidence: 0.799237034

00:08:38.508 --> 00:08:41.406 what we did is we used an

NOTE Confidence: 0.799237034

00:08:41.406 --> 00:08:43.212 immunocompromised mouse that has

NOTE Confidence: 0.799237034

00:08:43.212 --> 00:08:45.840 transgenic expression of human Illinois 2,

NOTE Confidence: 0.799237034

00:08:45.840 --> 00:08:47.800 which of course is required

NOTE Confidence: 0.799237034

00:08:47.800 --> 00:08:50.240 for T cell survival in vivo.

NOTE Confidence: 0.799237034

00:08:50.240 --> 00:08:52.991 So into these animals we could implant

NOTE Confidence: 0.799237034

00:08:52.991 --> 00:08:55.564 either the parental or the, you know,

NOTE Confidence: 0.799237034

00:08:55.564 --> 00:08:57.674 genetically modified cancer cell lines

NOTE Confidence: 0.799237034

00:08:57.674 --> 00:09:00.009 and then adoptively transfer the mouse

NOTE Confidence: 0.799237034

00:09:00.009 --> 00:09:02.476 with the patient's own tilts, right.

NOTE Confidence: 0.799237034

00:09:02.476 --> 00:09:05.170 So we could study this interaction

NOTE Confidence: 0.799237034

00:09:05.252 --> 00:09:07.040 in vivo and as you can see,

NOTE Confidence: 0.799237034

00:09:07.040 --> 00:09:09.928 the tumors that had the CD 58 loss

NOTE Confidence: 0.799237034

00:09:09.928 --> 00:09:11.960 were completely resistant to ACT.

NOTE Confidence: 0.799237034

00:09:11.960 --> 00:09:14.702 They also had an approximately hundredfold

NOTE Confidence: 0.799237034

00:09:14.702 --> 00:09:17.000 lower infiltration with T cells,

NOTE Confidence: 0.799237034

00:09:17.000 --> 00:09:18.700 validating some of the predictions

NOTE Confidence: 0.799237034

00:09:18.700 --> 00:09:21.040 that we had made in patients.

NOTE Confidence: 0.799237034

00:09:21.040 --> 00:09:23.864 And all of these effects could be rescued

NOTE Confidence: 0.799237034

00:09:23.864 --> 00:09:26.880 by RE expressing CD58 in the cancer cell.

NOTE Confidence: 0.799237034

00:09:26.880 --> 00:09:28.932 So overall this suggested that loss

NOTE Confidence: 0.799237034

00:09:28.932 --> 00:09:31.226 of CD 58 on the cancer cell conferred

NOTE Confidence: 0.799237034

00:09:31.226 --> 00:09:32.840 impaired T cell infiltration,

NOTE Confidence: 0.799237034

00:09:32.840 --> 00:09:35.360 proliferation and resistance to ACT.

NOTE Confidence: 0.799237034

00:09:35.360 --> 00:09:37.754 So coming back to that interesting

NOTE Confidence: 0.799237034

00:09:37.754 --> 00:09:39.920 interaction which I mentioned earlier,

NOTE Confidence: 0.799237034

00:09:39.920 --> 00:09:44.400 this interaction between CD58 and PDL 1.

NOTE Confidence: 0.799237034

00:09:44.400 --> 00:09:47.878 So we did a very simple experiment

NOTE Confidence: 0.799237034

00:09:47.878 --> 00:09:49.836 in which we knocked out CD58 and

NOTE Confidence: 0.799237034

00:09:49.836 --> 00:09:51.782 simply asked how much PDL one is

NOTE Confidence: 0.799237034

00:09:51.782 --> 00:09:53.840 on the surface of these cells.

NOTE Confidence: 0.799237034

00:09:53.840 --> 00:09:56.366 And in fact, when we knocked out CD58,

NOTE Confidence: 0.799237034

00:09:56.366 --> 00:09:59.880 we found that these cells do have more CD 58,

NOTE Confidence: 0.799237034

00:09:59.880 --> 00:10:02.400 excuse me, PDL one protein on the surface.

NOTE Confidence: 0.799237034

00:10:02.400 --> 00:10:04.872 And this effect again could be

NOTE Confidence: 0.799237034

00:10:04.872 --> 00:10:08.200 rescued by RE expressing CD58 itself.

NOTE Confidence: 0.799237034

00:10:08.200 --> 00:10:09.600 So the question then is,

NOTE Confidence: 0.799237034

00:10:09.600 --> 00:10:12.288 you know what sort of regulates this

NOTE Confidence: 0.799237034

00:10:12.288 --> 00:10:14.957 interaction and how do you go about this?

NOTE Confidence: 0.799237034

00:10:14.960 --> 00:10:17.186 Because we there's no like nothing really

NOTE Confidence: 0.799237034

00:10:17.186 --> 00:10:20.040 to help us inform of where to even start,

NOTE Confidence: 0.799237034

00:10:20.040 --> 00:10:20.363 right.

NOTE Confidence: 0.799237034

00:10:20.363 --> 00:10:21.978 What we could exclude pretty

NOTE Confidence: 0.799237034

00:10:21.978 --> 00:10:24.475 quickly is that there was no direct

NOTE Confidence: 0.799237034

00:10:24.475 --> 00:10:25.999 interaction between the proteins.

NOTE Confidence: 0.799237034

00:10:26.000 --> 00:10:28.072 So there had to be some sort of

NOTE Confidence: 0.799237034

00:10:28.072 --> 00:10:29.520 mediator that regulates that.

NOTE Confidence: 0.799237034

00:10:29.520 --> 00:10:31.734 So to do this systematically we

NOTE Confidence: 0.799237034

00:10:31.734 --> 00:10:34.542 did a genome scale loss of function

NOTE Confidence: 0.799237034

00:10:34.542 --> 00:10:37.452 screen that would show us or point

NOTE Confidence: 0.799237034

00:10:37.452 --> 00:10:39.684 us towards genes or proteins that

NOTE Confidence: 0.799237034

00:10:39.684 --> 00:10:41.439 are required for this interaction.

NOTE Confidence: 0.799237034

00:10:41.440 --> 00:10:43.208 So the design of the screen was that

NOTE Confidence: 0.799237034

00:10:43.208 --> 00:10:45.117 we took these Melanoma cell lines that

NOTE Confidence: 0.799237034

00:10:45.117 --> 00:10:47.143 express CAS nine and then we introduced

NOTE Confidence: 0.799237034

00:10:47.143 --> 00:10:48.835 the genome scale guide library to

NOTE Confidence: 0.799237034

00:10:48.835 --> 00:10:51.040 knock on every gene in the genome,

NOTE Confidence: 0.799237034

00:10:51.040 --> 00:10:52.520 let the cells, you know,

NOTE Confidence: 0.799237034

00:10:52.520 --> 00:10:54.560 edit for a couple of weeks.

NOTE Confidence: 0.799237034

00:10:54.560 --> 00:10:57.584 And then we sorted out the CD 58

NOTE Confidence: 0.799237034

00:10:57.584 --> 00:11:00.237 negative or CD 58 positive cells,

NOTE Confidence: 0.799237034

00:11:00.240 --> 00:11:01.995 sequenced the guide RNH guide

NOTE Confidence: 0.799237034

00:11:01.995 --> 00:11:04.240 RNAs in each of these pools.

NOTE Confidence: 0.799237034

00:11:04.240 --> 00:11:05.956 And the premise here is that

NOTE Confidence: 0.799237034

00:11:05.956 --> 00:11:07.800 in the CD 58 low pool,

NOTE Confidence: 0.799237034

00:11:07.800 --> 00:11:10.404 there must be perturbations that are

NOTE Confidence: 0.799237034

00:11:10.404 --> 00:11:12.599 somehow involved in regulating CD 58.

NOTE Confidence: 0.799237034

00:11:12.600 --> 00:11:13.998 So when you lose that gene,

NOTE Confidence: 0.799237034

00:11:14.000 --> 00:11:15.885 you see a reduction in CD58.

NOTE Confidence: 0.799237034

00:11:15.885 --> 00:11:17.600 And this is precisely what we saw.

NOTE Confidence: 0.799237034

00:11:17.600 --> 00:11:20.722 So here is a result of that screen.

NOTE Confidence: 0.799237034

00:11:20.722 --> 00:11:21.136 Reassuringly,

NOTE Confidence: 0.799237034

00:11:21.136 --> 00:11:24.060 the top hit of the screen was

NOTE Confidence: 0.799237034

00:11:24.060 --> 00:11:25.880 knockout of CD 58 itself,

NOTE Confidence: 0.799237034

00:11:25.880 --> 00:11:26.680 knockout CD58.

NOTE Confidence: 0.799237034

00:11:26.680 --> 00:11:29.258 There will be CD50 negative and a

NOTE Confidence: 0.799237034

00:11:29.258 --> 00:11:30.236 bunch of others.

NOTE Confidence: 0.799237034

00:11:30.240 --> 00:11:31.836 The one that really caught our attention,

NOTE Confidence: 0.799237034

00:11:31.840 --> 00:11:34.204 but we because we saw it

NOTE Confidence: 0.799237034

00:11:34.204 --> 00:11:35.780 also to physically interact

NOTE Confidence: 0.867276469090909

00:11:35.857 --> 00:11:38.480 with CD58IN in a mass spec Co IP screen,

NOTE Confidence: 0.867276469090909

00:11:38.480 --> 00:11:40.664 is this gene or protein called

NOTE Confidence: 0.867276469090909

00:11:40.664 --> 00:11:42.120 CMTM 6 super interesting?

NOTE Confidence: 0.867276469090909

00:11:42.120 --> 00:11:44.520 I know David had actually done

NOTE Confidence: 0.867276469090909

00:11:44.520 --> 00:11:46.560 some work that looked at the

NOTE Confidence: 0.867276469090909

00:11:46.560 --> 00:11:47.960 prognostic value of this protein,

NOTE Confidence: 0.867276469090909

00:11:47.960 --> 00:11:49.976 but it was unclear and I think we

NOTE Confidence: 0.867276469090909

00:11:49.976 --> 00:11:51.890 have the answer to why that might

NOTE Confidence: 0.867276469090909

00:11:51.890 --> 00:11:54.360 be So what was really interesting,

NOTE Confidence: 0.867276469090909

00:11:54.360 --> 00:11:56.600 just, you know, a year or two

NOTE Confidence: 0.867276469090909

00:11:56.600 --> 00:11:59.278 before we had made this observation,

NOTE Confidence: 0.867276469090909

00:11:59.280 --> 00:12:02.022 there were two Nature papers published

NOTE Confidence: 0.867276469090909

00:12:02.022 --> 00:12:05.009 showing that the same gene or protein

NOTE Confidence: 0.867276469090909

00:12:05.009 --> 00:12:07.515 CMTM 6 was in fact required for

NOTE Confidence: 0.867276469090909

00:12:07.598 --> 00:12:10.874 maintaining PDL one protein on the surface.

NOTE Confidence: 0.867276469090909

00:12:10.880 --> 00:12:13.504 So this was a plausible, you know,

NOTE Confidence: 0.867276469090909

00:12:13.504 --> 00:12:15.216 logical sort of hit to to go

NOTE Confidence: 0.867276469090909

00:12:15.216 --> 00:12:16.640 after and this is what we did.

NOTE Confidence: 0.867276469090909

00:12:16.640 --> 00:12:18.800 So when we knockout CMTM 6,

NOTE Confidence: 0.867276469090909

00:12:18.800 --> 00:12:22.240 we see a reduction in both CD58 and

NOTE Confidence: 0.867276469090909

00:12:22.240 --> 00:12:24.240 PDL one protein surface abundance.

NOTE Confidence: 0.867276469090909

00:12:24.240 --> 00:12:26.640 And when you rescue the gene CMTM 6,

NOTE Confidence: 0.867276469090909

00:12:26.640 --> 00:12:28.592 you rescue that and you have you you

NOTE Confidence: 0.867276469090909

00:12:28.592 --> 00:12:30.518 you bring them back to the baseline.

NOTE Confidence: 0.867276469090909

00:12:30.520 --> 00:12:32.188 And to really prove that this

NOTE Confidence: 0.867276469090909

00:12:32.188 --> 00:12:34.040 is required for the interaction,

NOTE Confidence: 0.867276469090909

00:12:34.040 --> 00:12:36.410 we generated a number of additional

NOTE Confidence: 0.867276469090909

00:12:36.410 --> 00:12:37.200 genetic perturbations,

NOTE Confidence: 0.867276469090909

00:12:37.200 --> 00:12:39.534 double mutants which we rescued where

NOTE Confidence: 0.867276469090909

00:12:39.534 --> 00:12:42.500 we rescued only one gene at a time

NOTE Confidence: 0.867276469090909

00:12:42.500 --> 00:12:44.504 where we could in fact demonstrate

NOTE Confidence: 0.867276469090909

00:12:44.581 --> 00:12:47.323 that CMTM 6 was required for

NOTE Confidence: 0.867276469090909

00:12:47.323 --> 00:12:49.151 mediating this reciprocal interaction.

NOTE Confidence: 0.867276469090909

00:12:49.160 --> 00:12:51.085 You know the the issue with these

NOTE Confidence: 0.867276469090909

00:12:51.085 --> 00:12:52.912 types of down signals, right,

NOTE Confidence: 0.867276469090909

00:12:52.912 --> 00:12:54.092 is always, well,

NOTE Confidence: 0.867276469090909

00:12:54.092 --> 00:12:56.084 how do you think about making

NOTE Confidence: 0.867276469090909

00:12:56.084 --> 00:12:57.480 a therapy from that,

NOTE Confidence: 0.867276469090909

00:12:57.480 --> 00:12:57.811 right?

NOTE Confidence: 0.867276469090909

00:12:57.811 --> 00:12:59.797 Because ultimately that's always the goal.

NOTE Confidence: 0.867276469090909

00:12:59.800 --> 00:13:01.928 So I'm going to skip a lot of

NOTE Confidence: 0.867276469090909

00:13:01.928 --> 00:13:03.958 data that we show in the paper.

NOTE Confidence: 0.867276469090909

00:13:03.960 --> 00:13:05.832 But Long story short,

NOTE Confidence: 0.867276469090909

00:13:05.832 --> 00:13:09.243 we identify we found that the binding

NOTE Confidence: 0.867276469090909

00:13:09.243 --> 00:13:12.955 A sequences on CD58 and PDL one for

NOTE Confidence: 0.867276469090909

00:13:12.955 --> 00:13:15.987 CM takes actually CMTM 6 actually

NOTE Confidence: 0.867276469090909

00:13:15.987 --> 00:13:18.260 differ and so we imagined that

NOTE Confidence: 0.867276469090909

00:13:18.260 --> 00:13:19.800 we could leverage that knowledge.

NOTE Confidence: 0.867276469090909

00:13:19.800 --> 00:13:24.328 So it turns out that CMTM 6 binds

NOTE Confidence: 0.867276469090909

00:13:24.328 --> 00:13:28.360 to a specific amino acid domain

NOTE Confidence: 0.867276469090909

00:13:28.360 --> 00:13:31.688 on North terminal domain in PDL 1

NOTE Confidence: 0.867276469090909

00:13:31.688 --> 00:13:34.600 spanning the amino acids 20 to 32.

NOTE Confidence: 0.867276469090909

00:13:34.600 --> 00:13:36.436 So when we scramble that area,

NOTE Confidence: 0.867276469090909

00:13:36.440 --> 00:13:37.720 turns out that, you know,

NOTE Confidence: 0.867276469090909

00:13:37.720 --> 00:13:41.320 CMTM 6 can no longer bind to PDL 1.

NOTE Confidence: 0.867276469090909

00:13:41.320 --> 00:13:44.416 So our we imagined that if we could

NOTE Confidence: 0.867276469090909

00:13:44.416 --> 00:13:45.962 selectively disrupt the interaction

NOTE Confidence: 0.867276469090909

00:13:45.962 --> 00:13:49.200 between PDL one and CMTM 6 at that site,

NOTE Confidence: 0.867276469090909

00:13:49.200 --> 00:13:51.944 it should result in reduction in PDL

NOTE Confidence: 0.867276469090909

00:13:51.944 --> 00:13:54.597 1 without affecting the levels of CD 58.

NOTE Confidence: 0.867276469090909

00:13:54.600 --> 00:13:56.560 And this is precisely what we saw.

NOTE Confidence: 0.867276469090909

00:13:56.560 --> 00:13:58.114 So we took these Melanoma cell lines,

NOTE Confidence: 0.867276469090909

00:13:58.120 --> 00:13:59.680 we knocked out PDL One,

NOTE Confidence: 0.867276469090909

00:13:59.680 --> 00:14:02.228 and then we rescued either the wild

NOTE Confidence: 0.867276469090909

00:14:02.228 --> 00:14:04.924 type orph or a orph where we scrambled

NOTE Confidence: 0.867276469090909

00:14:04.924 --> 00:14:07.964 that region that is unable to bind CMTM 6.

NOTE Confidence: 0.867276469090909

00:14:07.964 --> 00:14:10.465 And as you can see, the WILD type

NOTE Confidence: 0.867276469090909

00:14:10.465 --> 00:14:12.440 orph rescues PDL ONE expression,

NOTE Confidence: 0.867276469090909

00:14:12.440 --> 00:14:15.160 but the mutant does not.

NOTE Confidence: 0.867276469090909

00:14:15.160 --> 00:14:16.558 And then in a Co-op experiment,

NOTE Confidence: 0.867276469090909

00:14:16.560 --> 00:14:19.502 we could also directly show that this

NOTE Confidence: 0.867276469090909

00:14:19.502 --> 00:14:22.274 variant where we scramble that sequence

NOTE Confidence: 0.867276469090909

00:14:22.274 --> 00:14:25.030 is unable to has a significantly

NOTE Confidence: 0.867276469090909

00:14:25.030 --> 00:14:28.033 lower binding of Co-op to CMTM 6.

NOTE Confidence: 0.867276469090909

00:14:28.040 --> 00:14:31.238 So just to summarize this part,

NOTE Confidence: 0.867276469090909

00:14:31.240 --> 00:14:33.256 I hope I was able to show you that

NOTE Confidence: 0.867276469090909

00:14:33.256 --> 00:14:35.572 we were able to go from you know

NOTE Confidence: 0.867276469090909

00:14:35.572 --> 00:14:37.624 sequencing data to using the right

NOTE Confidence: 0.867276469090909

00:14:37.624 --> 00:14:39.479 functional tools to really inform

NOTE Confidence: 0.929347958571429

00:14:39.480 --> 00:14:42.077 precisely kind of what to go after.

NOTE Confidence: 0.929347958571429

00:14:42.080 --> 00:14:43.781 But you know what we were left

NOTE Confidence: 0.929347958571429

00:14:43.781 --> 00:14:45.239 with is actually the question,

NOTE Confidence: 0.929347958571429

00:14:45.240 --> 00:14:47.320 how many of these interactions do we miss,

NOTE Confidence: 0.929347958571429

00:14:47.320 --> 00:14:49.342 right. Every time you knockout a

NOTE Confidence: 0.929347958571429

00:14:49.342 --> 00:14:51.520 gene and you observe A phenotype,

NOTE Confidence: 0.929347958571429

00:14:51.520 --> 00:14:53.200 you know how do we know that

NOTE Confidence: 0.929347958571429

00:14:53.200 --> 00:14:54.620 that's not mediated through a

NOTE Confidence: 0.929347958571429

00:14:54.620 --> 00:14:55.920 number of these interactions.

NOTE Confidence: 0.929347958571429

00:14:55.920 --> 00:14:58.080 And the, the the clinical or

NOTE Confidence: 0.929347958571429

00:14:58.080 --> 00:14:59.708 therapeutic correlate of that is,

NOTE Confidence: 0.929347958571429

00:14:59.708 --> 00:15:01.610 you know giving somebody a single

NOTE Confidence: 0.929347958571429

00:15:01.672 --> 00:15:03.891 agent immunotherapy or you know even 2

NOTE Confidence: 0.929347958571429

00:15:03.891 --> 00:15:06.000 drugs and asking well what do these,

NOTE Confidence: 0.929347958571429

00:15:06.000 --> 00:15:08.121 what does inhibition of these two proteins

NOTE Confidence: 0.929347958571429

00:15:08.121 --> 00:15:10.157 do to everything else that's going on,

NOTE Confidence: 0.929347958571429

00:15:10.160 --> 00:15:12.600 on the surface or within within the cell.

NOTE Confidence: 0.929347958571429

00:15:12.600 --> 00:15:15.216 And this is actually something we're

NOTE Confidence: 0.929347958571429

00:15:15.216 --> 00:15:16.960 trying to address systematically.

NOTE Confidence: 0.929347958571429

00:15:16.960 --> 00:15:17.271 OK.

NOTE Confidence: 0.929347958571429

00:15:17.271 --> 00:15:19.759 So switching gears a little bit and coming

NOTE Confidence: 0.929347958571429

00:15:19.759 --> 00:15:22.355 to the second chapter which is a much,

NOTE Confidence: 0.929347958571429

00:15:22.360 --> 00:15:25.168 much more recent chapter in the

NOTE Confidence: 0.929347958571429

00:15:25.168 --> 00:15:28.252 lab that is leveraging novel

NOTE Confidence: 0.929347958571429

00:15:28.252 --> 00:15:31.517 base editing tools to hopefully

NOTE Confidence: 0.929347958571429

00:15:31.517 --> 00:15:35.038 improve cell based immunotherapies.

NOTE Confidence: 0.929347958571429

00:15:35.040 --> 00:15:37.824 So all of you know that cell based

NOTE Confidence: 0.929347958571429

00:15:37.824 --> 00:15:39.766 immunotherapies are now a critical

NOTE Confidence: 0.929347958571429

00:15:39.766 --> 00:15:42.124 component of the treatment of many

NOTE Confidence: 0.929347958571429

00:15:42.124 --> 00:15:43.765 hematologic malignancies and most

NOTE Confidence: 0.929347958571429

00:15:43.765 --> 00:15:46.123 recently there was also an approval

NOTE Confidence: 0.929347958571429

00:15:46.123 --> 00:15:48.570 for the the treatment of till

NOTE Confidence: 0.929347958571429

00:15:48.570 --> 00:15:50.292 transfer for patients with Melanoma.

NOTE Confidence: 0.929347958571429

00:15:50.292 --> 00:15:52.014 And you know the the typical you

NOTE Confidence: 0.929347958571429

00:15:52.014 --> 00:15:53.706 know workflow is so that you take

NOTE Confidence: 0.929347958571429

00:15:53.706 --> 00:15:55.188 something out of the patient and

NOTE Confidence: 0.929347958571429

00:15:55.188 --> 00:15:56.694 you don't do something with the

NOTE Confidence: 0.929347958571429

00:15:56.694 --> 00:15:58.436 cells and you you put them back in.

NOTE Confidence: 0.929347958571429

00:15:58.440 --> 00:15:59.960 And in the context of CAR T cells,

NOTE Confidence: 0.929347958571429

00:15:59.960 --> 00:16:02.130 of course that's taking PBMCS and putting

NOTE Confidence: 0.929347958571429

00:16:02.130 --> 00:16:04.917 a CAR into the cells and reinfusing them.

NOTE Confidence: 0.929347958571429

00:16:04.920 --> 00:16:07.476 In the context of till therapy,

NOTE Confidence: 0.929347958571429

00:16:07.480 --> 00:16:08.940 it is isolating tills

NOTE Confidence: 0.929347958571429

00:16:08.940 --> 00:16:10.035 from metastatic lesions,

NOTE Confidence: 0.929347958571429

00:16:10.040 --> 00:16:11.534 expand them ex vivo and then

NOTE Confidence: 0.929347958571429

00:16:11.534 --> 00:16:13.403 give them back to the patient so

NOTE Confidence: 0.929347958571429

00:16:13.403 --> 00:16:14.758 that they have in common.

NOTE Confidence: 0.929347958571429

00:16:14.760 --> 00:16:16.640 What they also have in common is the,

NOTE Confidence: 0.929347958571429

00:16:16.640 --> 00:16:18.560 the observation that has really

NOTE Confidence: 0.929347958571429

00:16:18.560 --> 00:16:21.297 emerged in the last few years is

NOTE Confidence: 0.929347958571429

00:16:21.297 --> 00:16:23.887 that there are very specific T cell

NOTE Confidence: 0.929347958571429

00:16:23.887 --> 00:16:26.183 features before you put this therapy

NOTE Confidence: 0.929347958571429

00:16:26.183 --> 00:16:28.430 into the patient that are strongly

NOTE Confidence: 0.929347958571429

00:16:28.430 --> 00:16:30.770 predictive of whether or not that

NOTE Confidence: 0.929347958571429

00:16:30.770 --> 00:16:32.958 cell product is going to work.

NOTE Confidence: 0.929347958571429

00:16:32.960 --> 00:16:34.850 And and this was published by the

NOTE Confidence: 0.929347958571429

00:16:34.850 --> 00:16:36.613 Rosenberg Group A few years ago

NOTE Confidence: 0.929347958571429

00:16:36.613 --> 00:16:38.098 and there's nothing shocking about

NOTE Confidence: 0.929347958571429

00:16:38.098 --> 00:16:39.600 some of the observation.

NOTE Confidence: 0.929347958571429

00:16:39.600 --> 00:16:42.624 But it really is sort of the rationale

NOTE Confidence: 0.929347958571429

00:16:42.624 --> 00:16:45.305 for thinking about how we can improve

NOTE Confidence: 0.929347958571429

00:16:45.305 --> 00:16:47.658 T cell function itself to build

NOTE Confidence: 0.929347958571429

00:16:47.658 --> 00:16:50.196 better cell cell therapies on top.

NOTE Confidence: 0.929347958571429

00:16:50.200 --> 00:16:50.908 And of course,

NOTE Confidence: 0.929347958571429

00:16:50.908 --> 00:16:52.560 we're not the only ones thinking about.

NOTE Confidence: 0.929347958571429

00:16:52.560 --> 00:16:54.648 There's a lot of groups take CAR T

NOTE Confidence: 0.929347958571429

00:16:54.648 --> 00:16:56.215 cells until therapies and engineer

NOTE Confidence: 0.929347958571429

00:16:56.215 --> 00:16:58.518 them in a number of different ways.

NOTE Confidence: 0.929347958571429

00:16:58.520 --> 00:16:59.066 You know,

NOTE Confidence: 0.929347958571429

00:16:59.066 --> 00:17:00.704 frequently in the last few years

NOTE Confidence: 0.929347958571429

00:17:00.704 --> 00:17:02.612 what people have done is, you know,

NOTE Confidence: 0.929347958571429

00:17:02.612 --> 00:17:03.264 for example,

NOTE Confidence: 0.929347958571429

00:17:03.264 --> 00:17:05.306 knocking out an inhibitory receptor

NOTE Confidence: 0.929347958571429

00:17:05.306 --> 00:17:07.413 such as CTLA 4, right?

NOTE Confidence: 0.929347958571429

00:17:07.413 --> 00:17:09.184 And I picked one study from Carl

NOTE Confidence: 0.929347958571429

00:17:09.184 --> 00:17:10.639 Jun's group could have picked,

NOTE Confidence: 0.929347958571429

00:17:10.640 --> 00:17:11.312 you know,

NOTE Confidence: 0.929347958571429

00:17:11.312 --> 00:17:13.202 hundreds of other papers where they

NOTE Confidence: 0.929347958571429

00:17:13.202 --> 00:17:15.668 try to improve CAR T cell therapy by

NOTE Confidence: 0.929347958571429

00:17:15.668 --> 00:17:17.964 deleting some of these inhibitory receptors.

NOTE Confidence: 0.929347958571429

00:17:17.964 --> 00:17:20.784 But the challenge with knocking

NOTE Confidence: 0.929347958571429

00:17:20.784 --> 00:17:24.044 out a gene especially in T cells

NOTE Confidence: 0.929347958571429

00:17:24.044 --> 00:17:26.628 is that one of the off target

NOTE Confidence: 0.929347958571429

00:17:26.628 --> 00:17:28.198 effects of CRISPR CAS 9,

NOTE Confidence: 0.929347958571429

00:17:28.200 --> 00:17:30.210 which is due to the double

NOTE Confidence: 0.929347958571429

00:17:30.210 --> 00:17:31.550 stranded DNA breaks that

NOTE Confidence: 0.929933964166667

00:17:31.622 --> 00:17:33.530 take place is that you actually get

NOTE Confidence: 0.929933964166667

00:17:33.530 --> 00:17:35.440 a pretty high rate of aneuploidy.

NOTE Confidence: 0.929933964166667

00:17:35.440 --> 00:17:39.081 So 7 to 14% of cells in of T cells in

NOTE Confidence: 0.929933964166667

00:17:39.081 --> 00:17:41.640 a pool that you you know engineer with

NOTE Confidence: 0.929933964166667

00:17:41.640 --> 00:17:43.440 CRISPR CAS nine will be anemployed.

NOTE Confidence: 0.929933964166667

00:17:43.440 --> 00:17:45.786 And of course aneuploidy or chromosomal

NOTE Confidence: 0.929933964166667

00:17:45.786 --> 00:17:48.043 instability as I will talk about

NOTE Confidence: 0.929933964166667

00:17:48.043 --> 00:17:50.234 later is is a hallmark of cancer.

NOTE Confidence: 0.929933964166667

00:17:50.240 --> 00:17:52.760 And that comes with all sorts of concerns.

NOTE Confidence: 0.929933964166667

00:17:52.760 --> 00:17:55.700 Of course, what got me really

NOTE Confidence: 0.929933964166667

00:17:55.700 --> 00:17:57.530 interested in thinking about how to

NOTE Confidence: 0.929933964166667

00:17:57.593 --> 00:17:59.148 improve cell therapies are papers

NOTE Confidence: 0.929933964166667

00:17:59.148 --> 00:18:01.441 like the one that I'm showing you

NOTE Confidence: 0.929933964166667

00:18:01.441 --> 00:18:03.475 here from the Jonathan Powell group.

NOTE Confidence: 0.929933964166667

00:18:03.480 --> 00:18:05.811 So rather than deleting a gene or

NOTE Confidence: 0.929933964166667

00:18:05.811 --> 00:18:08.026 over expressing a gene which which

NOTE Confidence: 0.929933964166667

00:18:08.026 --> 00:18:10.360 comes with other issues, you know,

NOTE Confidence: 0.929933964166667

00:18:10.360 --> 00:18:12.360 they made a really interesting

NOTE Confidence: 0.929933964166667

00:18:12.360 --> 00:18:14.772 observation that is they found that

NOTE Confidence: 0.929933964166667

00:18:14.772 --> 00:18:17.052 some of their mice had sporadic

NOTE Confidence: 0.929933964166667

00:18:17.052 --> 00:18:19.035 mutations in a gene called TSE 2.

NOTE Confidence: 0.929933964166667

00:18:19.040 --> 00:18:20.260 The mutation itself really

NOTE Confidence: 0.929933964166667

00:18:20.260 --> 00:18:21.480 doesn't matter so much.

NOTE Confidence: 0.929933964166667

00:18:21.480 --> 00:18:24.135 But what they were able to show is actually

NOTE Confidence: 0.929933964166667

00:18:24.135 --> 00:18:26.439 when you take T cells from the mouse

NOTE Confidence: 0.929933964166667

00:18:26.439 --> 00:18:28.911 that has this germline mutation and you

NOTE Confidence: 0.929933964166667

00:18:28.911 --> 00:18:31.275 adoptively transfer mice with the wild

NOTE Confidence: 0.929933964166667

00:18:31.280 --> 00:18:33.758 type gene that harbor Melanoma tumors,

NOTE Confidence: 0.929933964166667

00:18:33.760 --> 00:18:36.118 those mutant T cells are much,

NOTE Confidence: 0.929933964166667

00:18:36.120 --> 00:18:38.815 much more potent in eliminating not only

NOTE Confidence: 0.929933964166667

00:18:38.815 --> 00:18:41.391 melanomas but they also show this in

NOTE Confidence: 0.929933964166667

00:18:41.391 --> 00:18:44.080 context of leukemias and and other diseases.

NOTE Confidence: 0.929933964166667

00:18:44.080 --> 00:18:46.054 So what that suggested to us is

NOTE Confidence: 0.929933964166667

00:18:46.054 --> 00:18:47.839 that maybe we don't have to,

NOTE Confidence: 0.929933964166667

00:18:47.840 --> 00:18:48.320 you know,

NOTE Confidence: 0.929933964166667

00:18:48.320 --> 00:18:49.760 take the wheels off the car.

NOTE Confidence: 0.929933964166667

00:18:49.760 --> 00:18:52.112 I mean knocking out an entire gene with

NOTE Confidence: 0.929933964166667

00:18:52.112 --> 00:18:54.559 all of its unintended consequences,

NOTE Confidence: 0.929933964166667

00:18:54.560 --> 00:18:57.320 maybe it's sufficient to introduce very

NOTE Confidence: 0.929933964166667

00:18:57.320 --> 00:19:00.140 specific mutations in genes that will

NOTE Confidence: 0.929933964166667

00:19:00.140 --> 00:19:02.400 significantly alter T cell function.

NOTE Confidence: 0.929933964166667

00:19:02.400 --> 00:19:04.506 And this is the hypothesis that

NOTE Confidence: 0.929933964166667

00:19:04.506 --> 00:19:07.085 we sought to to test in this

NOTE Confidence: 0.929933964166667

00:19:07.085 --> 00:19:09.275 project that just like in mice,

NOTE Confidence: 0.929933964166667

00:19:09.280 --> 00:19:12.059 there must be either naturally a cure

NOTE Confidence: 0.929933964166667

00:19:12.059 --> 00:19:14.154 occurring or synthetic protein variants

NOTE Confidence: 0.929933964166667

00:19:14.154 --> 00:19:17.073 that may enhance T cell function and

NOTE Confidence: 0.929933964166667

00:19:17.073 --> 00:19:19.580 therefore may enable the production of

NOTE Confidence: 0.929933964166667

00:19:19.580 --> 00:19:23.320 more effective cell therapies on top.

NOTE Confidence: 0.929933964166667

00:19:23.320 --> 00:19:25.468 And the method that you know

NOTE Confidence: 0.929933964166667

00:19:25.468 --> 00:19:27.559 would be required to do that,

NOTE Confidence: 0.929933964166667

00:19:27.560 --> 00:19:29.247 One of the ways to do that

NOTE Confidence: 0.929933964166667

00:19:29.247 --> 00:19:30.520 is using base editors.

NOTE Confidence: 0.929933964166667

00:19:30.520 --> 00:19:33.474 So there's different types of base editors.

NOTE Confidence: 0.929933964166667

00:19:33.480 --> 00:19:38.901 These are CRISPR CAS 9 dependent base

NOTE Confidence: 0.929933964166667

00:19:38.901 --> 00:19:42.147 editors that either citadine or adenosine

NOTE Confidence: 0.929933964166667

00:19:42.147 --> 00:19:44.750 deaminase linked proteins that unlike

NOTE Confidence: 0.929933964166667

00:19:44.750 --> 00:19:47.456 CRISPR CAS 9 don't introduce double

NOTE Confidence: 0.929933964166667

00:19:47.456 --> 00:19:50.386 stranded DNA breaks but rather they

NOTE Confidence: 0.929933964166667

00:19:50.386 --> 00:19:52.826 induce deamination events in very

NOTE Confidence: 0.929933964166667

00:19:52.826 --> 00:19:56.160 specific windows guided by guide RNAs.

NOTE Confidence: 0.929933964166667

00:19:56.160 --> 00:19:58.960 And ultimately in the in the example of

NOTE Confidence: 0.929933964166667

00:19:58.960 --> 00:20:01.346 citadine base editors you get C to T

NOTE Confidence: 0.929933964166667

00:20:01.346 --> 00:20:03.480 changes and in the context of denizine

NOTE Confidence: 0.929933964166667

00:20:03.480 --> 00:20:06.040 base editors you get A to G changes.

NOTE Confidence: 0.929933964166667

00:20:06.040 --> 00:20:08.672 So what this allows you to do

NOTE Confidence: 0.929933964166667

00:20:08.672 --> 00:20:11.057 is introduce at some specificity

NOTE Confidence: 0.929933964166667

00:20:11.057 --> 00:20:14.264 mutations at defined loci in a gene

NOTE Confidence: 0.929933964166667

00:20:14.264 --> 00:20:15.992 rather than knocking out the gene.

NOTE Confidence: 0.929933964166667

00:20:16.000 --> 00:20:18.317 And one of the challenges that that

NOTE Confidence: 0.929933964166667

00:20:18.317 --> 00:20:20.543 had existed with these base editors

NOTE Confidence: 0.929933964166667

00:20:20.543 --> 00:20:22.877 was the issue that their efficiency,

NOTE Confidence: 0.929933964166667

00:20:22.880 --> 00:20:24.758 especially in primary human T cells,

NOTE Confidence: 0.929933964166667

00:20:24.760 --> 00:20:26.680 was rather low.

NOTE Confidence: 0.929933964166667

00:20:26.680 --> 00:20:29.976 So we This was led by an MDPHD

NOTE Confidence: 0.929933964166667

00:20:29.976 --> 00:20:31.320 student in my lab.

NOTE Confidence: 0.929933964166667

00:20:31.320 --> 00:20:33.240 Zach Walsh has shown all the

NOTE Confidence: 0.929933964166667

00:20:33.240 --> 00:20:34.322 way to your left.

NOTE Confidence: 0.929933964166667

00:20:34.322 --> 00:20:36.100 He took it upon himself to try

NOTE Confidence: 0.929933964166667

00:20:36.164 --> 00:20:37.924 to improve the efficiency of

NOTE Confidence: 0.929933964166667

00:20:37.924 --> 00:20:39.684 these base editors because these

NOTE Confidence: 0.903999559666667

00:20:39.751 --> 00:20:41.551 would be the right tools to

NOTE Confidence: 0.903999559666667

00:20:41.551 --> 00:20:43.120 really introduce some of these

NOTE Confidence: 0.903999559666667

00:20:43.120 --> 00:20:44.920 mutations in a targeted fashion.

NOTE Confidence: 0.903999559666667

00:20:44.920 --> 00:20:47.688 And he's done that through a really sort of

NOTE Confidence: 0.903999559666667

00:20:47.688 --> 00:20:50.516 smart way of delivering the base editor.

NOTE Confidence: 0.903999559666667

00:20:50.520 --> 00:20:52.392 I'm not going to go through all the details,

NOTE Confidence: 0.903999559666667

00:20:52.400 --> 00:20:54.576 but suffice it to say, you know,

NOTE Confidence: 0.903999559666667

00:20:54.576 --> 00:20:57.264 we're able to achieve extremely high

NOTE Confidence: 0.903999559666667

00:20:57.264 --> 00:20:58.946 efficiency, relatively speaking,

NOTE Confidence: 0.903999559666667

00:20:58.946 --> 00:21:03.228 between 80% and and 99% with these base

NOTE Confidence: 0.903999559666667

00:21:03.228 --> 00:21:05.513 editors introducing very precise variants.

NOTE Confidence: 0.903999559666667

00:21:05.520 --> 00:21:07.515 This is a paper that is accepted,

NOTE Confidence: 0.903999559666667

00:21:07.520 --> 00:21:09.758 that will be published next week.

NOTE Confidence: 0.903999559666667

00:21:09.760 --> 00:21:11.220 But so, you know,

NOTE Confidence: 0.903999559666667

00:21:11.220 --> 00:21:12.680 equipped with these methods,

NOTE Confidence: 0.903999559666667

00:21:12.680 --> 00:21:13.912 we then imagined, well,

NOTE Confidence: 0.903999559666667

00:21:13.912 --> 00:21:15.760 what do we want to actually,

NOTE Confidence: 0.903999559666667

00:21:15.760 --> 00:21:17.034 you know, edit in these T cells?

NOTE Confidence: 0.903999559666667

00:21:17.040 --> 00:21:18.360 What do you even start?

NOTE Confidence: 0.903999559666667

00:21:18.360 --> 00:21:20.232 We we can't do this on the genome scale.

NOTE Confidence: 0.903999559666667

00:21:20.240 --> 00:21:22.080 There's too many bases, right?

NOTE Confidence: 0.903999559666667

00:21:22.080 --> 00:21:24.678 It would be an impractical experiment.

NOTE Confidence: 0.903999559666667

00:21:24.680 --> 00:21:27.128 So what we decided to do is rather

NOTE Confidence: 0.903999559666667

00:21:27.128 --> 00:21:29.557 be guided by experiments of nature.

NOTE Confidence: 0.903999559666667

00:21:29.560 --> 00:21:31.037 And what I mean with that is,

NOTE Confidence: 0.903999559666667

00:21:31.040 --> 00:21:31.680 you know,

NOTE Confidence: 0.903999559666667

00:21:31.680 --> 00:21:33.920 there's a lot of variants out there

NOTE Confidence: 0.903999559666667

00:21:33.920 --> 00:21:36.513 that are reported to be either

NOTE Confidence: 0.903999559666667

00:21:36.513 --> 00:21:38.277 definitively associated with immune,

NOTE Confidence: 0.903999559666667

00:21:38.280 --> 00:21:39.885 clinical, immune syndromes,

NOTE Confidence: 0.903999559666667

00:21:39.885 --> 00:21:42.025 either autoimmunity or immunodeficiency

NOTE Confidence: 0.903999559666667

00:21:42.025 --> 00:21:44.960 and everything in between or variants.

NOTE Confidence: 0.903999559666667

00:21:44.960 --> 00:21:47.104 And these are most of them that are

NOTE Confidence: 0.903999559666667

00:21:47.104 --> 00:21:48.517 variants of uncertain significance

NOTE Confidence: 0.903999559666667

00:21:48.517 --> 00:21:50.959 where there may be an association,

NOTE Confidence: 0.903999559666667

00:21:50.960 --> 00:21:52.640 but we don't know exactly because

NOTE Confidence: 0.903999559666667

00:21:52.640 --> 00:21:54.239 we can't prove each of them,

NOTE Confidence: 0.903999559666667

00:21:54.240 --> 00:21:56.277 you know, emotionally one at a time.

NOTE Confidence: 0.903999559666667

00:21:56.280 --> 00:21:58.808 So what we decided to do is put

NOTE Confidence: 0.903999559666667

00:21:58.808 --> 00:22:01.268 together sort of a library of 30,000

NOTE Confidence: 0.903999559666667

00:22:01.268 --> 00:22:03.520 variants that are out there across

NOTE Confidence: 0.903999559666667

00:22:03.520 --> 00:22:06.363 102 genes spanning all major T

NOTE Confidence: 0.903999559666667

00:22:06.363 --> 00:22:07.978 cell functions and introduce all

NOTE Confidence: 0.903999559666667

00:22:07.978 --> 00:22:10.119 of them with these base editors

NOTE Confidence: 0.903999559666667

00:22:10.119 --> 00:22:12.079 in a massively parallel fashion.

NOTE Confidence: 0.903999559666667

00:22:12.080 --> 00:22:15.615 And then ask how each of these

NOTE Confidence: 0.903999559666667

00:22:15.615 --> 00:22:18.488 variants changes known hallmarks of

NOTE Confidence: 0.903999559666667

00:22:18.488 --> 00:22:22.040 T cell mediated anti tumor immunity

NOTE Confidence: 0.903999559666667

00:22:22.040 --> 00:22:24.356 including the activation of T cells,

NOTE Confidence: 0.903999559666667

00:22:24.360 --> 00:22:26.280 the proliferation, cytochrome production,

NOTE Confidence: 0.903999559666667

00:22:26.280 --> 00:22:28.536 long term expansion, persistence, etcetera.

NOTE Confidence: 0.903999559666667

00:22:28.536 --> 00:22:29.760 And the the,

NOTE Confidence: 0.903999559666667

00:22:29.760 --> 00:22:32.760 the premise here is that we want to

NOTE Confidence: 0.903999559666667

00:22:32.760 --> 00:22:34.669 identify variants from this pool

NOTE Confidence: 0.903999559666667

00:22:34.669 --> 00:22:36.835 that improve most and perhaps all

NOTE Confidence: 0.903999559666667

00:22:36.835 --> 00:22:38.969 of those favorable features that

NOTE Confidence: 0.903999559666667

00:22:38.969 --> 00:22:41.519 we know are important to build

NOTE Confidence: 0.903999559666667

00:22:41.520 --> 00:22:44.120 good cell therapies on top.

NOTE Confidence: 0.903999559666667

00:22:44.120 --> 00:22:46.248 And so just you know a word

NOTE Confidence: 0.903999559666667

00:22:46.248 --> 00:22:47.160 about negative control.

NOTE Confidence: 0.903999559666667

00:22:47.160 --> 00:22:48.290 So in addition to the

NOTE Confidence: 0.903999559666667

00:22:48.290 --> 00:22:49.194 variants that we introduced,

NOTE Confidence: 0.903999559666667

00:22:49.200 --> 00:22:50.630 we had a number of

NOTE Confidence: 0.903999559666667

00:22:50.630 --> 00:22:51.418 different negative controls.

NOTE Confidence: 0.903999559666667

00:22:51.418 --> 00:22:52.663 I think it's always important

NOTE Confidence: 0.903999559666667

00:22:52.663 --> 00:22:54.110 to think about these when you

NOTE Confidence: 0.903999559666667

00:22:54.110 --> 00:22:55.200 do these large scale screens.

NOTE Confidence: 0.903999559666667

00:22:55.200 --> 00:22:56.800 So here's a distribution and

NOTE Confidence: 0.903999559666667

00:22:56.800 --> 00:22:58.894 the log fold change of negative

NOTE Confidence: 0.903999559666667

00:22:58.894 --> 00:23:01.039 controls that we had introduced.

NOTE Confidence: 0.903999559666667

00:23:01.040 --> 00:23:03.240 So these include splice acceptor

NOTE Confidence: 0.903999559666667

00:23:03.240 --> 00:23:05.000 and splice donor variants.

NOTE Confidence: 0.903999559666667

00:23:05.000 --> 00:23:07.170 Or when you when you mutate these

NOTE Confidence: 0.903999559666667

00:23:07.170 --> 00:23:09.204 these splice sites then what you

NOTE Confidence: 0.903999559666667

00:23:09.204 --> 00:23:11.280 get is truncated proteins that get

NOTE Confidence: 0.903999559666667

00:23:11.280 --> 00:23:13.263 basically knocked out or lack of a

NOTE Confidence: 0.903999559666667

00:23:13.263 --> 00:23:15.220 better word or they get truncated

NOTE Confidence: 0.903999559666667

00:23:15.220 --> 00:23:17.920 proteins that that are non functional.

NOTE Confidence: 0.903999559666667

00:23:17.920 --> 00:23:20.531 So as you can see these are

NOTE Confidence: 0.903999559666667

00:23:20.531 --> 00:23:22.409 significantly depleted at a very

NOTE Confidence: 0.903999559666667

00:23:22.409 --> 00:23:24.119 high lock fault rate ratio,

NOTE Confidence: 0.903999559666667

00:23:24.120 --> 00:23:26.155 while mutations that introduce silent

NOTE Confidence: 0.903999559666667

00:23:26.155 --> 00:23:28.190 changes or empty window changes

NOTE Confidence: 0.903999559666667

00:23:28.255 --> 00:23:30.439 don't change the distribution at all.

NOTE Confidence: 0.903999559666667

00:23:30.440 --> 00:23:31.108 In addition,

NOTE Confidence: 0.903999559666667

00:23:31.108 --> 00:23:33.112 another good control for T cells

NOTE Confidence: 0.903999559666667

00:23:33.112 --> 00:23:34.958 of course is introducing mutations

NOTE Confidence: 0.903999559666667

00:23:34.958 --> 00:23:37.184 that result in disruption of the

NOTE Confidence: 0.903999559666667

00:23:37.184 --> 00:23:39.436 CD3 complex because the cells need

NOTE Confidence: 0.908319066153846

00:23:39.440 --> 00:23:41.344 the complex to to be activated and

NOTE Confidence: 0.908319066153846

00:23:41.344 --> 00:23:42.879 proliferate and do what they do.

NOTE Confidence: 0.908319066153846

00:23:42.880 --> 00:23:44.455 And these are also significantly

NOTE Confidence: 0.908319066153846

00:23:44.455 --> 00:23:46.449 depleted and and this was highly

NOTE Confidence: 0.908319066153846

00:23:46.449 --> 00:23:47.526 consistent between different

NOTE Confidence: 0.908319066153846

00:23:47.526 --> 00:23:49.680 donors that we did the screen,

NOTE Confidence: 0.908319066153846

00:23:49.680 --> 00:23:51.396 so we did them multiple donors.

NOTE Confidence: 0.908319066153846

00:23:51.400 --> 00:23:53.672 So here are a few results from that

NOTE Confidence: 0.908319066153846

00:23:53.672 --> 00:23:55.784 screen now and I'm going to show

NOTE Confidence: 0.908319066153846

00:23:55.784 --> 00:23:57.640 a couple of very selective ones.

NOTE Confidence: 0.908319066153846

00:23:57.640 --> 00:24:01.276 So here we are looking at the lock fold,

NOTE Confidence: 0.908319066153846

00:24:01.280 --> 00:24:04.290 a change of genes and the designated

NOTE Confidence: 0.908319066153846

00:24:04.290 --> 00:24:07.080 variants that were introduced and the

NOTE Confidence: 0.908319066153846

00:24:07.080 --> 00:24:09.376 you know the negative lock 10 FDR.

NOTE Confidence: 0.908319066153846

00:24:09.376 --> 00:24:12.384 So the higher you go the most statistically

NOTE Confidence: 0.908319066153846

00:24:12.384 --> 00:24:14.432 significant things were in the screen

NOTE Confidence: 0.908319066153846

00:24:14.432 --> 00:24:16.727 and again there were many sort of

NOTE Confidence: 0.908319066153846

00:24:16.727 --> 00:24:18.611 expected depleted genes such as CD3

NOTE Confidence: 0.908319066153846

00:24:18.611 --> 00:24:21.277 or you know row A and what have you.

NOTE Confidence: 0.908319066153846

00:24:21.280 --> 00:24:25.319 But what caught our attention was this

NOTE Confidence: 0.908319066153846

00:24:25.320 --> 00:24:28.038 number of these mutations that were

NOTE Confidence: 0.908319066153846

00:24:28.038 --> 00:24:30.555 enriched meaning they improved T cell

NOTE Confidence: 0.908319066153846

00:24:30.555 --> 00:24:32.767 function were found in the PIC three

NOTE Confidence: 0.908319066153846

00:24:32.767 --> 00:24:35.682 CD gene and also in the PIC 3R1 gene.

NOTE Confidence: 0.908319066153846

00:24:35.682 --> 00:24:39.210 These two genes encode for the two domains

NOTE Confidence: 0.908319066153846

00:24:39.302 --> 00:24:44.000 of the immune cells specific PI3K delta.

NOTE Confidence: 0.908319066153846

00:24:44.000 --> 00:24:46.400 And this is another way to look at is this

NOTE Confidence: 0.908319066153846

00:24:46.457 --> 00:24:48.793 time we're only looking at pick three CD,

NOTE Confidence: 0.908319066153846

00:24:48.800 --> 00:24:50.762 this time looking at the amino

NOTE Confidence: 0.908319066153846

00:24:50.762 --> 00:24:52.789 acid sequence from left to right

NOTE Confidence: 0.908319066153846

00:24:52.789 --> 00:24:54.751 and where each of these mutations

NOTE Confidence: 0.908319066153846

00:24:54.751 --> 00:24:56.831 that either enrich on the top or

NOTE Confidence: 0.908319066153846

00:24:56.831 --> 00:24:58.588 deplete in the screen on the bottom.

NOTE Confidence: 0.908319066153846

00:24:58.588 --> 00:24:59.558 And as you can see,

NOTE Confidence: 0.908319066153846

00:24:59.560 --> 00:25:02.236 there's a number of of enriched

NOTE Confidence: 0.908319066153846

00:25:02.236 --> 00:25:04.589 variants here that are associated

NOTE Confidence: 0.908319066153846

00:25:04.589 --> 00:25:07.584 with a favorable TC cell phenotype,

NOTE Confidence: 0.908319066153846

00:25:07.584 --> 00:25:10.874 you know spanning residues 524 to 529.

NOTE Confidence: 0.908319066153846

00:25:10.874 --> 00:25:13.760 But then this is also this other variant,

NOTE Confidence: 0.908319066153846

00:25:13.760 --> 00:25:17.000 the C416R that was strongly enriched.

NOTE Confidence: 0.908319066153846

00:25:17.000 --> 00:25:18.040 And on the flip side,

NOTE Confidence: 0.908319066153846

00:25:18.040 --> 00:25:20.168 we have a mutation that that causes

NOTE Confidence: 0.908319066153846

00:25:20.168 --> 00:25:22.640 a loss of function of this gene.

NOTE Confidence: 0.908319066153846

00:25:22.640 --> 00:25:24.572 When you look at where these

NOTE Confidence: 0.908319066153846

00:25:24.572 --> 00:25:27.092 where this all of these gain of

NOTE Confidence: 0.908319066153846

00:25:27.092 --> 00:25:28.620 function mutations are located,

NOTE Confidence: 0.908319066153846

00:25:28.620 --> 00:25:31.280 they're not anywhere in the kinase domain.

NOTE Confidence: 0.908319066153846

00:25:31.280 --> 00:25:32.844 As you might imagine,

NOTE Confidence: 0.908319066153846

00:25:32.844 --> 00:25:34.799 since this is the catalytic

NOTE Confidence: 0.908319066153846

00:25:34.799 --> 00:25:37.077 sort of subunit of PR3K delta,

NOTE Confidence: 0.908319066153846

00:25:37.080 --> 00:25:39.019 they are all aligned and this is

NOTE Confidence: 0.908319066153846

00:25:39.019 --> 00:25:40.520 a prediction from alpha fold.

NOTE Confidence: 0.908319066153846

00:25:40.520 --> 00:25:43.490 They're all aligned at the interface

NOTE Confidence: 0.908319066153846

00:25:43.490 --> 00:25:45.971 between these two gene products

NOTE Confidence: 0.908319066153846

00:25:45.971 --> 00:25:48.834 of pick three CD and pick 3R1.

NOTE Confidence: 0.908319066153846

00:25:48.840 --> 00:25:49.581 So you know,

NOTE Confidence: 0.908319066153846

00:25:49.581 --> 00:25:51.839 we of course then went on to to

NOTE Confidence: 0.908319066153846

00:25:51.839 --> 00:25:53.240 validate these observations.

NOTE Confidence: 0.908319066153846

00:25:53.240 --> 00:25:54.638 You know one of the expectations

NOTE Confidence: 0.908319066153846

00:25:54.638 --> 00:25:56.124 would be that you have higher

NOTE Confidence: 0.908319066153846

00:25:56.124 --> 00:25:57.960 output from the PI3K pathways.

NOTE Confidence: 0.908319066153846

00:25:57.960 --> 00:26:01.200 So we looked at downstream signalling

NOTE Confidence: 0.908319066153846

00:26:01.200 --> 00:26:03.209 at phosphor akt and phosphorus 6 and

NOTE Confidence: 0.908319066153846

00:26:03.209 --> 00:26:05.200 we tested many different variants.

NOTE Confidence: 0.908319066153846

00:26:05.200 --> 00:26:05.926 But I'm just,

NOTE Confidence: 0.908319066153846

00:26:05.926 --> 00:26:07.136 you know highlighting here in

NOTE Confidence: 0.908319066153846

00:26:07.136 --> 00:26:08.899 the red box the loss of function

NOTE Confidence: 0.908319066153846

00:26:08.899 --> 00:26:10.455 mutation and then the green box

NOTE Confidence: 0.908319066153846

00:26:10.455 --> 00:26:11.800 the gain of function mutation.

NOTE Confidence: 0.908319066153846

00:26:11.800 --> 00:26:13.519 And as you can see the gain of function,

NOTE Confidence: 0.908319066153846

00:26:13.520 --> 00:26:15.984 you see more phosphor AKT and more

NOTE Confidence: 0.908319066153846

00:26:15.984 --> 00:26:18.543 phosphorus 6 while it is both of these

NOTE Confidence: 0.908319066153846

00:26:18.543 --> 00:26:20.839 are reduced in the loss of function.

NOTE Confidence: 0.908319066153846

00:26:20.840 --> 00:26:22.681 In the same vein and again I

NOTE Confidence: 0.908319066153846

00:26:22.681 --> 00:26:24.017 highlighted that with the green

NOTE Confidence: 0.908319066153846

00:26:24.017 --> 00:26:25.746 boxes the gain of function and in

NOTE Confidence: 0.908319066153846

00:26:25.746 --> 00:26:27.675 the red boxes the loss of function.

NOTE Confidence: 0.908319066153846

00:26:27.680 --> 00:26:29.192 And this is just a selection of the data.

NOTE Confidence: 0.908319066153846

00:26:29.200 --> 00:26:31.076 But we can see that the gain

NOTE Confidence: 0.908319066153846

00:26:31.076 --> 00:26:31.880 of function variant

NOTE Confidence: 0.8518369175

00:26:31.880 --> 00:26:34.172 was associated with improved TNF alpha

NOTE Confidence: 0.8518369175

00:26:34.172 --> 00:26:36.079 production and proliferation and so on.

NOTE Confidence: 0.8518369175

00:26:36.080 --> 00:26:37.640 You know an initial validation

NOTE Confidence: 0.8518369175

00:26:37.640 --> 00:26:39.200 of the of the screen.

NOTE Confidence: 0.8518369175

00:26:39.200 --> 00:26:40.532 So now of course the question

NOTE Confidence: 0.8518369175

00:26:40.532 --> 00:26:42.240 is can we use this information

NOTE Confidence: 0.8518369175

00:26:42.240 --> 00:26:43.760 and improve cell therapies?

NOTE Confidence: 0.8518369175

00:26:43.760 --> 00:26:46.434 Can they be better of cell killers?

NOTE Confidence: 0.8518369175

00:26:46.440 --> 00:26:48.472 And to do this we used a simple

NOTE Confidence: 0.8518369175

00:26:48.472 --> 00:26:50.157 coke culture experiment like the one

NOTE Confidence: 0.8518369175

00:26:50.157 --> 00:26:52.528 that I had presented to you earlier.

NOTE Confidence: 0.8518369175

00:26:52.528 --> 00:26:55.220 Only this time we engineered the T

NOTE Confidence: 0.8518369175

00:26:55.220 --> 00:26:57.523 cells to express a very specific T

NOTE Confidence: 0.8518369175

00:26:57.523 --> 00:26:59.639 cell receptor against Nye cell one,

NOTE Confidence: 0.8518369175

00:26:59.640 --> 00:27:01.725 which is a commonly expressed

NOTE Confidence: 0.8518369175

00:27:01.725 --> 00:27:03.393 neo antigen on Melanoma.

NOTE Confidence: 0.8518369175

00:27:03.400 --> 00:27:05.675 So we can really test the specificity

NOTE Confidence: 0.8518369175

00:27:05.675 --> 00:27:08.380 and then we either use the native T

NOTE Confidence: 0.8518369175

00:27:08.380 --> 00:27:11.081 cells or we introduced one of a number

NOTE Confidence: 0.8518369175

00:27:11.081 --> 00:27:13.356 of variants that we had identified in

NOTE Confidence: 0.8518369175

00:27:13.360 --> 00:27:15.439 the screen and then Co culture them.

NOTE Confidence: 0.8518369175

00:27:15.440 --> 00:27:17.472 And as you can see again the gain

NOTE Confidence: 0.8518369175

00:27:17.472 --> 00:27:19.304 of function in green was strongly

NOTE Confidence: 0.8518369175

00:27:19.304 --> 00:27:21.623 associated with a higher degree of Poly

NOTE Confidence: 0.8518369175

00:27:21.623 --> 00:27:23.428 functionality here summarized as the

NOTE Confidence: 0.8518369175

00:27:23.428 --> 00:27:25.792 fraction of cells that expressed TNF alpha,

NOTE Confidence: 0.8518369175

00:27:25.792 --> 00:27:28.374 renzon B and IL 2 while the loss

NOTE Confidence: 0.8518369175

00:27:28.374 --> 00:27:30.279 of function showed a reduction.

NOTE Confidence: 0.8518369175

00:27:30.280 --> 00:27:32.626 And then this also translated in

NOTE Confidence: 0.8518369175

00:27:32.626 --> 00:27:34.640 improved license of Melanoma cells.

NOTE Confidence: 0.8518369175

00:27:34.640 --> 00:27:36.998 So here I think you can see my cursor.

NOTE Confidence: 0.8518369175

00:27:37.000 --> 00:27:37.239 Yep.

NOTE Confidence: 0.8518369175

00:27:37.239 --> 00:27:38.912 So here is the gain of function

NOTE Confidence: 0.8518369175

00:27:38.912 --> 00:27:40.685 variant and we're looking at the

NOTE Confidence: 0.8518369175

00:27:40.685 --> 00:27:42.235 number of surviving Melanoma cells.

NOTE Confidence: 0.8518369175

00:27:42.240 --> 00:27:44.396 As you can see that strongly reduced

NOTE Confidence: 0.8518369175

00:27:44.400 --> 00:27:47.358 the loss of function does not

NOTE Confidence: 0.8518369175

00:27:47.360 --> 00:27:50.237 enhance the activity of the T cells.

NOTE Confidence: 0.8518369175

00:27:50.240 --> 00:27:51.655 What was really gratifying and

NOTE Confidence: 0.8518369175

00:27:51.655 --> 00:27:53.346 this sort of closing the loop

NOTE Confidence: 0.8518369175

00:27:53.346 --> 00:27:54.879 to the first part of my talk,

NOTE Confidence: 0.8518369175

00:27:54.880 --> 00:27:57.416 we were also able to show that this

NOTE Confidence: 0.8518369175

00:27:57.416 --> 00:28:00.573 gain of function variant was able to

NOTE Confidence: 0.8518369175

00:28:00.573 --> 00:28:02.494 overcome resistance from CD58 loss.

NOTE Confidence: 0.8518369175

00:28:02.494 --> 00:28:04.310 So we did all of these coke culture

NOTE Confidence: 0.8518369175

00:28:04.366 --> 00:28:06.172 experiments, all repeated them,

NOTE Confidence: 0.8518369175

00:28:06.172 --> 00:28:09.340 only this time we knocked out CD58 and

NOTE Confidence: 0.8518369175

00:28:09.340 --> 00:28:11.854 then the coke culture and the C416R,

NOTE Confidence: 0.8518369175

00:28:11.854 --> 00:28:13.912 the game function variant and T cells

NOTE Confidence: 0.8518369175

00:28:13.912 --> 00:28:16.377 was in fact able to almost completely

NOTE Confidence: 0.8518369175

00:28:16.377 --> 00:28:18.192 radical themselves and you know,

NOTE Confidence: 0.8518369175

00:28:18.200 --> 00:28:20.279 without the labouring the point too much.

NOTE Confidence: 0.8518369175

00:28:20.280 --> 00:28:22.296 We also tested the same strategy in

NOTE Confidence: 0.8518369175

00:28:22.296 --> 00:28:24.695 a number of different CAR T cells and

NOTE Confidence: 0.8518369175

00:28:24.695 --> 00:28:26.783 we find the exact same thing whether

NOTE Confidence: 0.8518369175

00:28:26.783 --> 00:28:29.252 you use the CD9 CAR or CD22 CAR

NOTE Confidence: 0.8518369175

00:28:29.252 --> 00:28:30.876 against different leukemia models.

NOTE Confidence: 0.8518369175

00:28:30.880 --> 00:28:32.932 Introducing these variants in the T

NOTE Confidence: 0.8518369175

00:28:32.932 --> 00:28:35.898 cell that is the basis for making that

NOTE Confidence: 0.8518369175

00:28:35.898 --> 00:28:37.639 product improved their functionality

NOTE Confidence: 0.8518369175

00:28:37.639 --> 00:28:40.357 and their ability to lyse these.

NOTE Confidence: 0.8518369175

00:28:40.360 --> 00:28:43.640 Looking as summarise this portion

NOTE Confidence: 0.8518369175

00:28:43.640 --> 00:28:44.951 of the presentation,

NOTE Confidence: 0.8518369175

00:28:44.951 --> 00:28:48.405 Hope was able to show you that we

NOTE Confidence: 0.8518369175

00:28:48.405 --> 00:28:50.960 are now able to base edit primary

NOTE Confidence: 0.8518369175

00:28:50.960 --> 00:28:54.040 human T cells with a high efficiency

NOTE Confidence: 0.8518369175

00:28:54.040 --> 00:28:57.000 that unbiased discovery of variants

NOTE Confidence: 0.8518369175

00:28:57.000 --> 00:28:59.272 from a from a big pool of variants

NOTE Confidence: 0.8518369175

00:28:59.272 --> 00:29:01.480 may be able to identify those that

NOTE Confidence: 0.8518369175

00:29:01.480 --> 00:29:03.640 improve T cell function and those

NOTE Confidence: 0.8518369175

00:29:03.640 --> 00:29:05.968 perhaps could be used to improve cell

NOTE Confidence: 0.8518369175

00:29:05.968 --> 00:29:07.720 therapies broadly in the future.

NOTE Confidence: 0.86158250875

00:29:13.400 --> 00:29:17.726 OK. So now I'm going to switch to a

NOTE Confidence: 0.86158250875

00:29:17.726 --> 00:29:20.008 somewhat different area of the of the

NOTE Confidence: 0.86158250875

00:29:20.008 --> 00:29:23.075 lab or work in the lab that we're doing.

NOTE Confidence: 0.86158250875

00:29:23.080 --> 00:29:25.840 But you know the common theme is that we are

NOTE Confidence: 0.86158250875

00:29:25.909 --> 00:29:28.234 interested in what causes immune evasion

NOTE Confidence: 0.86158250875

00:29:28.234 --> 00:29:30.936 and and lack of response to immunotherapies.

NOTE Confidence: 0.86158250875

00:29:30.936 --> 00:29:34.200 And the the reason we got into this,

NOTE Confidence: 0.86158250875

00:29:34.200 --> 00:29:35.360 again it's a clinical one.

NOTE Confidence: 0.86158250875

00:29:35.360 --> 00:29:37.278 As you as all of you know,

NOTE Confidence: 0.86158250875

00:29:37.280 --> 00:29:39.605 brain metastasis are a common

NOTE Confidence: 0.86158250875

00:29:39.605 --> 00:29:41.000 problem across cancers,

NOTE Confidence: 0.86158250875

00:29:41.000 --> 00:29:42.840 but very common in Melanoma.

NOTE Confidence: 0.86158250875

00:29:42.840 --> 00:29:45.837 In fact, the incidence is probably as high as

NOTE Confidence: 0.86158250875

00:29:45.840 --> 00:29:49.676 75% in patients who have advanced disease.

NOTE Confidence: 0.86158250875

00:29:49.680 --> 00:29:51.655 And while the combination of

NOTE Confidence: 0.86158250875

00:29:51.655 --> 00:29:53.235 immunotherapies are you know,

NOTE Confidence: 0.86158250875

00:29:53.240 --> 00:29:57.160 showing efficacy against brain metastasis,

NOTE Confidence: 0.86158250875

00:29:57.160 --> 00:29:58.520 there's still a lot of work to do.

NOTE Confidence: 0.86158250875

00:29:58.520 --> 00:30:00.472 You know one of the reasons for that

NOTE Confidence: 0.86158250875

00:30:00.472 --> 00:30:02.733 is that those regimens are very toxic

NOTE Confidence: 0.86158250875

00:30:02.733 --> 00:30:04.910 and and despite the activity in some

NOTE Confidence: 0.86158250875

00:30:04.973 --> 00:30:07.318 patients we we still see you know

NOTE Confidence: 0.86158250875

00:30:07.318 --> 00:30:09.304 forms of Immunivision that that seem

NOTE Confidence: 0.86158250875

00:30:09.304 --> 00:30:11.439 to be pretty distinct in the brain.

NOTE Confidence: 0.86158250875

00:30:11.440 --> 00:30:13.794 So the the you know the the motivation

NOTE Confidence: 0.86158250875

00:30:13.794 --> 00:30:16.293 was really to study a brain metastasis

NOTE Confidence: 0.86158250875

00:30:16.293 --> 00:30:19.084 but I'll show you how that sort of got us

NOTE Confidence: 0.86158250875

00:30:19.084 --> 00:30:21.222 into this field of chromosomal instability.

NOTE Confidence: 0.86158250875

00:30:21.222 --> 00:30:24.710 So a couple years ago we we published a

NOTE Confidence: 0.86158250875

00:30:24.710 --> 00:30:27.491 paper in this was led by Jana Johannes

NOTE Confidence: 0.86158250875

00:30:27.491 --> 00:30:31.030 and Yiping postdocs in my lab where we

NOTE Confidence: 0.86158250875

00:30:31.030 --> 00:30:32.800 asked a simple question in patients,

NOTE Confidence: 0.86158250875

00:30:32.800 --> 00:30:35.008 what is the difference between an

NOTE Confidence: 0.86158250875

00:30:35.008 --> 00:30:37.080 untreated brain metastasis and an

NOTE Confidence: 0.86158250875

00:30:37.080 --> 00:30:38.679 untreated extracranial metastasis.

NOTE Confidence: 0.86158250875

00:30:38.680 --> 00:30:41.072 What we didn't want is any sort of

NOTE Confidence: 0.86158250875

00:30:41.072 --> 00:30:42.280 therapeutic intervention in between.

NOTE Confidence: 0.86158250875

00:30:42.280 --> 00:30:44.230 We're really interested just in

NOTE Confidence: 0.86158250875

00:30:44.230 --> 00:30:46.670 the salient biology which has been

NOTE Confidence: 0.86158250875

00:30:46.670 --> 00:30:48.780 quite poorly described actually in

NOTE Confidence: 0.86158250875

00:30:48.780 --> 00:30:51.194 patients compared to other you know

NOTE Confidence: 0.86158250875

00:30:51.194 --> 00:30:52.839 areas in Melanoma at least.

NOTE Confidence: 0.86158250875

00:30:52.840 --> 00:30:55.027 And I just want to point out a couple

NOTE Confidence: 0.86158250875

00:30:55.027 --> 00:30:57.236 of sort of results from this paper.

NOTE Confidence: 0.86158250875

00:30:57.236 --> 00:31:00.546 The first one is when we compare Melanoma

NOTE Confidence: 0.86158250875

00:31:00.546 --> 00:31:04.116 brain Mets MBM versus extracranial Mets ECM,

NOTE Confidence: 0.86158250875

00:31:04.120 --> 00:31:06.437 we found that the brain Mets were

NOTE Confidence: 0.86158250875

00:31:06.437 --> 00:31:08.884 had a higher fraction of the genome

NOTE Confidence: 0.86158250875

00:31:08.884 --> 00:31:12.697 altered FGA and that is a surrogate or a

NOTE Confidence: 0.86158250875

00:31:12.697 --> 00:31:14.917 process called chromosomal instability.

NOTE Confidence: 0.86158250875

00:31:14.920 --> 00:31:16.336 What is chromosomal instability?

NOTE Confidence: 0.86158250875

00:31:16.336 --> 00:31:18.760 It is a a a hallmark of cancer.

NOTE Confidence: 0.86158250875

00:31:18.760 --> 00:31:21.935 It's rather broadly seen across

NOTE Confidence: 0.86158250875

00:31:21.935 --> 00:31:24.237 almost every solid tumor.

NOTE Confidence: 0.86158250875

00:31:24.237 --> 00:31:27.413 And one of the ways by which chromosomal

NOTE Confidence: 0.86158250875

00:31:27.413 --> 00:31:29.804 instability can arise is through errors

NOTE Confidence: 0.86158250875

00:31:29.804 --> 00:31:32.400 that cancer cells make during anaphase,

NOTE Confidence: 0.86158250875

00:31:32.400 --> 00:31:34.280 where they don't segregate chromosomes

NOTE Confidence: 0.86158250875

00:31:34.280 --> 00:31:36.759 properly so that one of the data

NOTE Confidence: 0.86158250875

00:31:36.759 --> 00:31:38.895 cell you know is left with more and

NOTE Confidence: 0.86158250875

00:31:38.957 --> 00:31:40.955 the other one with less material.

NOTE Confidence: 0.86158250875

00:31:40.960 --> 00:31:42.955 The end product of this is aneuploidy,

NOTE Confidence: 0.86158250875

00:31:42.960 --> 00:31:44.396 right, and chromosomal civility,

NOTE Confidence: 0.86158250875

00:31:44.396 --> 00:31:46.191 sort of the perpetual dynamic

NOTE Confidence: 0.86158250875

00:31:46.191 --> 00:31:47.837 process that gives rise to that.

NOTE Confidence: 0.86158250875

00:31:47.840 --> 00:31:50.560 The extra material in the one of the

NOTE Confidence: 0.86158250875

00:31:50.560 --> 00:31:52.419 data cells is frequently packaged,

NOTE Confidence: 0.86158250875

00:31:52.419 --> 00:31:53.598 if it survives,

NOTE Confidence: 0.86158250875

00:31:53.600 --> 00:31:56.000 is frequently packaged in so-called

NOTE Confidence: 0.86158250875

00:31:56.000 --> 00:31:56.400 micronuclei.

NOTE Confidence: 0.86158250875

00:31:56.400 --> 00:31:59.856 So one of the ways to quantify chromosomal

NOTE Confidence: 0.86158250875

00:31:59.856 --> 00:32:01.968 instability more functionally beyond

NOTE Confidence: 0.86158250875

00:32:01.968 --> 00:32:04.924 just genomics is actually look at

NOTE Confidence: 0.86158250875

00:32:04.924 --> 00:32:06.874 the frequency of those micronuclei.

NOTE Confidence: 0.86158250875

00:32:06.880 --> 00:32:08.116 And this is what we did.

NOTE Confidence: 0.86158250875

00:32:08.120 --> 00:32:10.192 This is in the same study where we

NOTE Confidence: 0.86158250875

00:32:10.192 --> 00:32:12.258 had cell lines that were derived from

NOTE Confidence: 0.86158250875

00:32:12.258 --> 00:32:14.292 either a brain or an extracranial

NOTE Confidence: 0.86158250875

00:32:14.292 --> 00:32:16.757 metastasis from the same individual.

NOTE Confidence: 0.870622878

00:32:16.760 --> 00:32:18.536 We enumerated the rate of micronuclei

NOTE Confidence: 0.870622878

00:32:18.536 --> 00:32:20.999 and as you can see the one from the

NOTE Confidence: 0.870622878

00:32:20.999 --> 00:32:22.592 brain in fact had more micronuclei

NOTE Confidence: 0.870622878

00:32:22.592 --> 00:32:24.434 compared to the one that came

NOTE Confidence: 0.870622878

00:32:24.434 --> 00:32:26.392 from a lymph node in this case.

NOTE Confidence: 0.870622878

00:32:26.392 --> 00:32:28.828 And when we put these cells back into

NOTE Confidence: 0.870622878

00:32:28.828 --> 00:32:30.476 animals in immunocompromised mice,

NOTE Confidence: 0.870622878

00:32:30.480 --> 00:32:34.400 those cells in fact are more likely to

NOTE Confidence: 0.870622878

00:32:34.400 --> 00:32:36.395 cause brain metastasis in the mouse than

NOTE Confidence: 0.870622878

00:32:36.395 --> 00:32:38.479 those that come from outside the brain.

NOTE Confidence: 0.870622878

00:32:38.480 --> 00:32:40.286 The second sort of a key result

NOTE Confidence: 0.870622878

00:32:40.286 --> 00:32:42.895 from the study when we looked at the

NOTE Confidence: 0.870622878

00:32:42.895 --> 00:32:44.311 microenvironment was the observation

NOTE Confidence: 0.870622878

00:32:44.311 --> 00:32:46.644 that brain that appeared to have a much

NOTE Confidence: 0.870622878

00:32:46.644 --> 00:32:48.087 more rhotomogenic myelod compartment

NOTE Confidence: 0.870622878

00:32:48.087 --> 00:32:51.227 as you can see both from the single

NOTE Confidence: 0.870622878

00:32:51.227 --> 00:32:53.240 cell data to the on the left here.

NOTE Confidence: 0.870622878

00:32:53.240 --> 00:32:55.160 And then also we validated this

NOTE Confidence: 0.870622878

00:32:55.160 --> 00:32:57.192 in two independent patient cohorts

NOTE Confidence: 0.870622878

00:32:57.192 --> 00:32:58.800 by Multiplex immunofluorescence.

NOTE Confidence: 0.870622878

00:32:58.800 --> 00:33:01.999 So this is all in in Melanoma.

NOTE Confidence: 0.870622878

00:33:02.000 --> 00:33:04.368 The question then of course is you know

NOTE Confidence: 0.870622878

00:33:04.368 --> 00:33:06.543 what about other common cancers that

NOTE Confidence: 0.870622878

00:33:06.543 --> 00:33:09.240 frequently you know metastasize to the brain,

NOTE Confidence: 0.870622878

00:33:09.240 --> 00:33:11.207 The most common one in terms of

NOTE Confidence: 0.870622878

00:33:11.207 --> 00:33:13.437 prevalence is non small cell lung cancer.

NOTE Confidence: 0.870622878

00:33:13.440 --> 00:33:14.880 So naturally we're interested in

NOTE Confidence: 0.870622878

00:33:14.880 --> 00:33:16.918 asking do some of these concepts also

NOTE Confidence: 0.870622878

00:33:16.918 --> 00:33:18.822 apply to non small cell lung cancer

NOTE Confidence: 0.870622878

00:33:18.822 --> 00:33:20.753 And the answer is and this is sort of

NOTE Confidence: 0.870622878

00:33:20.753 --> 00:33:23.445 in an analogous study that we're that

NOTE Confidence: 0.870622878

00:33:23.445 --> 00:33:26.240 we are trying to publish right now.

NOTE Confidence: 0.870622878

00:33:26.240 --> 00:33:28.284 We also asked the same question in

NOTE Confidence: 0.870622878

00:33:28.284 --> 00:33:30.253 in that disease and this time though

NOTE Confidence: 0.870622878

00:33:30.253 --> 00:33:31.795 we had a lot more data.

NOTE Confidence: 0.870622878

00:33:31.800 --> 00:33:34.565 This time we could leverage data from

NOTE Confidence: 0.870622878

00:33:34.565 --> 00:33:37.434 the MSK impact cohort where we had

NOTE Confidence: 0.870622878

00:33:37.434 --> 00:33:40.346 genomic data that was linked with the

NOTE Confidence: 0.870622878

00:33:40.346 --> 00:33:42.600 location where the of the of the disease.

NOTE Confidence: 0.870622878

00:33:42.600 --> 00:33:44.511 So there's lots of primary tumors and

NOTE Confidence: 0.870622878

00:33:44.511 --> 00:33:46.255 then a bunch of different metastatic

NOTE Confidence: 0.870622878

00:33:46.255 --> 00:33:48.954 sites and then all the way on the right

NOTE Confidence: 0.870622878

00:33:48.954 --> 00:33:51.137 here you see again brain metastasis have

NOTE Confidence: 0.870622878

00:33:51.137 --> 00:33:53.159 the highest fraction of genome altered,

NOTE Confidence: 0.870622878

00:33:53.160 --> 00:33:55.104 again a surrogate for

NOTE Confidence: 0.870622878

00:33:55.104 --> 00:33:56.076 chromosomal instability.

NOTE Confidence: 0.870622878

00:33:56.080 --> 00:33:58.019 We went on to validate this and

NOTE Confidence: 0.870622878

00:33:58.019 --> 00:33:59.950 a couple of additional published

NOTE Confidence: 0.870622878

00:33:59.950 --> 00:34:02.680 cohorts that are out there as well

NOTE Confidence: 0.870622878

00:34:02.680 --> 00:34:05.800 as in a very large cohort of nearly

NOTE Confidence: 0.870622878

00:34:05.800 --> 00:34:07.684 9500 patients where we had whole

NOTE Confidence: 0.870622878

00:34:07.684 --> 00:34:09.472 exome and RNA sequencing Through

NOTE Confidence: 0.870622878

00:34:09.472 --> 00:34:10.879 an industry collaboration,

NOTE Confidence: 0.870622878

00:34:10.880 --> 00:34:12.518 we find the exact same observation

NOTE Confidence: 0.870622878

00:34:12.518 --> 00:34:14.324 that brain Mets are more unstable

NOTE Confidence: 0.870622878

00:34:14.324 --> 00:34:15.969 than extracranial Mets which are

NOTE Confidence: 0.870622878

00:34:15.969 --> 00:34:17.920 more unstable than the primary tumor.

NOTE Confidence: 0.870622878

00:34:17.920 --> 00:34:18.412 So.

NOTE Confidence: 0.870622878

00:34:18.412 --> 00:34:21.856 So clearly this process seems to be

NOTE Confidence: 0.870622878

00:34:21.856 --> 00:34:24.920 important in conferring a sort of

NOTE Confidence: 0.870622878

00:34:24.920 --> 00:34:26.600 aggressive phenotype and perhaps

NOTE Confidence: 0.870622878

00:34:26.600 --> 00:34:28.836 also in modulating the immune

NOTE Confidence: 0.870622878

00:34:28.836 --> 00:34:31.134 environment in an unfavorable way.

NOTE Confidence: 0.870622878

00:34:31.134 --> 00:34:33.378 But it's kind of an obscure

NOTE Confidence: 0.870622878

00:34:33.378 --> 00:34:35.555 concept to to study, right,

NOTE Confidence: 0.870622878

00:34:35.555 --> 00:34:37.930 because it's such a perpetual

NOTE Confidence: 0.870622878

00:34:37.930 --> 00:34:38.880 dynamic process.

NOTE Confidence: 0.870622878

00:34:38.880 --> 00:34:39.756 How do you,

NOTE Confidence: 0.870622878

00:34:39.756 --> 00:34:42.160 how do you go about actually studying that?

NOTE Confidence: 0.870622878

00:34:42.160 --> 00:34:44.197 I think the so the first question

NOTE Confidence: 0.870622878

00:34:44.200 --> 00:34:46.391 that we as ourselves is well what

NOTE Confidence: 0.870622878

00:34:46.391 --> 00:34:49.158 is a good model to use to study this

NOTE Confidence: 0.870622878

00:34:49.158 --> 00:34:52.299 and what is a good model that we we

NOTE Confidence: 0.870622878

00:34:52.299 --> 00:34:54.601 could sort of easily identify, right.

NOTE Confidence: 0.870622878

00:34:54.601 --> 00:34:57.809 So the way we approach this problem is

NOTE Confidence: 0.870622878

00:34:57.809 --> 00:35:01.366 we looked at public data again TCGAACR genie,

NOTE Confidence: 0.870622878

00:35:01.366 --> 00:35:04.187 CP tag and we asked which subsets

NOTE Confidence: 0.870622878

00:35:04.187 --> 00:35:08.038 of lung non small cell lung cancer

NOTE Confidence: 0.870622878

00:35:08.038 --> 00:35:09.721 are particularly chromosomally

NOTE Confidence: 0.870622878

00:35:09.721 --> 00:35:12.785 unstable and are defined by very

NOTE Confidence: 0.870622878

00:35:12.785 --> 00:35:14.237 distinct genomic subsets.

NOTE Confidence: 0.866529672333333

00:35:14.240 --> 00:35:17.103 And it turns out that one particular

NOTE Confidence: 0.866529672333333

00:35:17.103 --> 00:35:20.036 mutation or loss in a gene called LKB

NOTE Confidence: 0.866529672333333

00:35:20.036 --> 00:35:22.968 one also known as STK 11 was across

NOTE Confidence: 0.866529672333333

00:35:22.968 --> 00:35:25.422 the board associated with a higher

NOTE Confidence: 0.866529672333333

00:35:25.422 --> 00:35:26.983 rate of chromosomal instability.

NOTE Confidence: 0.866529672333333

00:35:26.983 --> 00:35:29.650 What you also need to know about

NOTE Confidence: 0.866529672333333

00:35:29.718 --> 00:35:31.926 this particular subset of non small

NOTE Confidence: 0.866529672333333

00:35:31.926 --> 00:35:34.040 cell lung cancer which is common

NOTE Confidence: 0.866529672333333

00:35:34.040 --> 00:35:36.550 is that these patients virtually

NOTE Confidence: 0.866529672333333

00:35:36.550 --> 00:35:38.558 never respond to immunotherapy.

NOTE Confidence: 0.866529672333333

00:35:38.560 --> 00:35:40.611 So this is from a paper published

NOTE Confidence: 0.866529672333333

00:35:40.611 --> 00:35:42.237 from MD Anderson where they

NOTE Confidence: 0.866529672333333

00:35:42.237 --> 00:35:44.277 looked at patients with or without

NOTE Confidence: 0.866529672333333

00:35:44.277 --> 00:35:46.197 mutations or deletions in SDK 11.

NOTE Confidence: 0.866529672333333

00:35:46.200 --> 00:35:47.800 And as you can see here in red,

NOTE Confidence: 0.866529672333333

00:35:47.800 --> 00:35:50.340 these patients do extremely poorly

NOTE Confidence: 0.866529672333333

00:35:50.340 --> 00:35:52.880 in response to PD1 inhibition.

NOTE Confidence: 0.866529672333333

00:35:52.880 --> 00:35:53.936 And lastly,

NOTE Confidence: 0.866529672333333

00:35:53.936 --> 00:35:56.048 this particular subset happens

NOTE Confidence: 0.866529672333333

00:35:56.048 --> 00:35:58.712 to also more frequently be

NOTE Confidence: 0.866529672333333

00:35:58.712 --> 00:36:00.920 associated with brain metastasis.

NOTE Confidence: 0.866529672333333

00:36:00.920 --> 00:36:03.356 So you know, with this information,

NOTE Confidence: 0.866529672333333

00:36:03.360 --> 00:36:06.656 we believe that this particular subset is a

NOTE Confidence: 0.866529672333333

00:36:06.656 --> 00:36:10.038 really good archetypical sinhi chromosomally,

NOTE Confidence: 0.866529672333333

00:36:10.040 --> 00:36:10.870 chromosomal instability,

NOTE Confidence: 0.866529672333333

00:36:10.870 --> 00:36:14.190 high disease to study some of those concepts

NOTE Confidence: 0.866529672333333

00:36:14.253 --> 00:36:16.598 that that we want to understand better.

NOTE Confidence: 0.866529672333333

00:36:16.600 --> 00:36:18.040 And this is what Lindsay,

NOTE Confidence: 0.866529672333333

00:36:18.040 --> 00:36:21.155 another MDPHD student in my Lac lab,

NOTE Confidence: 0.866529672333333

00:36:21.160 --> 00:36:22.672 took upon herself a couple of

NOTE Confidence: 0.866529672333333

00:36:22.672 --> 00:36:24.717 years ago and she started with a

NOTE Confidence: 0.866529672333333

00:36:24.717 --> 00:36:26.357 couple of very simple experiments.

NOTE Confidence: 0.866529672333333

00:36:26.360 --> 00:36:28.523 We brought in a few human cell

NOTE Confidence: 0.866529672333333

00:36:28.523 --> 00:36:30.465 lines which are shown here and

NOTE Confidence: 0.866529672333333

00:36:30.465 --> 00:36:32.320 two of them are LKB 1 deficient,

NOTE Confidence: 0.866529672333333

00:36:32.320 --> 00:36:34.918 the other one LKB 1 proficient.

NOTE Confidence: 0.866529672333333

00:36:34.920 --> 00:36:37.356 And we did imaging and enumerated

NOTE Confidence: 0.866529672333333

00:36:37.356 --> 00:36:40.080 the rates of these micronuclear.

NOTE Confidence: 0.866529672333333

00:36:40.080 --> 00:36:42.397 I'm showing you here 2 exemplary ones.

NOTE Confidence: 0.866529672333333

00:36:42.400 --> 00:36:43.888 And as we would predict from

NOTE Confidence: 0.866529672333333

00:36:43.888 --> 00:36:44.632 the genomic data,

NOTE Confidence: 0.866529672333333

00:36:44.640 --> 00:36:46.845 the LKB 1 deficient subset in fact

NOTE Confidence: 0.866529672333333

00:36:46.845 --> 00:36:49.159 had more of these micronuclear,

NOTE Confidence: 0.866529672333333

00:36:49.160 --> 00:36:51.290 suggesting that there are in fact

NOTE Confidence: 0.866529672333333

00:36:51.290 --> 00:36:52.355 more chromosomally unstable.

NOTE Confidence: 0.866529672333333

00:36:52.360 --> 00:36:55.656 We also got 2 cell lines from Quoc

NOTE Confidence: 0.866529672333333

00:36:55.656 --> 00:36:58.520 Wong at NYU where he had, you know,

NOTE Confidence: 0.866529672333333

00:36:58.520 --> 00:37:01.200 established the the KP model,

NOTE Confidence: 0.866529672333333

00:37:01.200 --> 00:37:03.132 you know which have the K Ras

NOTE Confidence: 0.866529672333333

00:37:03.132 --> 00:37:04.440 mutation and P53 mutant.

NOTE Confidence: 0.866529672333333

00:37:04.440 --> 00:37:06.464 But on top of that they had developed

NOTE Confidence: 0.866529672333333

00:37:06.464 --> 00:37:08.499 a model with deletion of LKB one

NOTE Confidence: 0.866529672333333

00:37:08.499 --> 00:37:10.360 and derived A syngenic cell lines.

NOTE Confidence: 0.866529672333333

00:37:10.360 --> 00:37:12.637 So we got those into the lab as well

NOTE Confidence: 0.866529672333333

00:37:12.637 --> 00:37:14.998 and find that the LKB 1 deficient line

NOTE Confidence: 0.866529672333333

00:37:14.998 --> 00:37:17.719 in fact was more chromosomally unstable.

NOTE Confidence: 0.866529672333333

00:37:17.720 --> 00:37:20.023 So this seems to be shared between

NOTE Confidence: 0.866529672333333

00:37:20.023 --> 00:37:22.376 both the human and the available

NOTE Confidence: 0.866529672333333

00:37:22.376 --> 00:37:23.978 best available mouse models.

NOTE Confidence: 0.866529672333333

00:37:23.978 --> 00:37:26.561 So now coming back for a second

NOTE Confidence: 0.866529672333333

00:37:26.561 --> 00:37:28.400 to these micronuclei,

NOTE Confidence: 0.866529672333333

00:37:28.400 --> 00:37:30.608 as I mentioned earlier they are

NOTE Confidence: 0.866529672333333

00:37:30.608 --> 00:37:32.895 you know extra material that are

NOTE Confidence: 0.866529672333333

00:37:32.895 --> 00:37:34.760 engulfed in these mini nuclei.

NOTE Confidence: 0.866529672333333

00:37:34.760 --> 00:37:35.660 But the the,

NOTE Confidence: 0.866529672333333

00:37:35.660 --> 00:37:37.160 the envelope of these micronuclei

NOTE Confidence: 0.866529672333333

00:37:37.160 --> 00:37:38.599 is very rupture prone.

NOTE Confidence: 0.866529672333333

00:37:38.600 --> 00:37:41.015 So the DNA within those is released

NOTE Confidence: 0.866529672333333

00:37:41.015 --> 00:37:43.568 at some rate into the cytosol which

NOTE Confidence: 0.866529672333333

00:37:43.568 --> 00:37:46.115 of course is an absolute no go

NOTE Confidence: 0.866529672333333

00:37:46.115 --> 00:37:48.320 when it comes to you know normal

NOTE Confidence: 0.866529672333333

00:37:48.320 --> 00:37:50.114 immunity where you know our bodies

NOTE Confidence: 0.866529672333333

00:37:50.114 --> 00:37:51.980 and this is highly conserved are

NOTE Confidence: 0.866529672333333

00:37:51.980 --> 00:37:54.115 trained to sense DNA in the cytosol

NOTE Confidence: 0.866529672333333

00:37:54.115 --> 00:37:56.057 from all sorts of infections for

NOTE Confidence: 0.866529672333333

00:37:56.057 --> 00:37:57.637 example and respond to that.

NOTE Confidence: 0.866529672333333

00:37:57.640 --> 00:38:00.840 And and you know cells do that very

NOTE Confidence: 0.866529672333333

00:38:00.840 --> 00:38:02.341 efficiently through several pathways,

NOTE Confidence: 0.866529672333333

00:38:02.341 --> 00:38:04.176 perhaps the most important one

NOTE Confidence: 0.866529672333333

00:38:04.176 --> 00:38:06.079 being the C gas sting pathway.

NOTE Confidence: 0.866529672333333

00:38:06.080 --> 00:38:10.910 So here C gas senses cytosolic DNA

NOTE Confidence: 0.866529672333333

00:38:10.910 --> 00:38:14.960 and well OK lights off convert,

NOTE Confidence: 0.866529672333333

00:38:14.960 --> 00:38:17.424 convert this DNA to C gam which binds

NOTE Confidence: 0.866529672333333

00:38:17.424 --> 00:38:19.808 to sting and ultimately triggers a

NOTE Confidence: 0.866529672333333

00:38:19.808 --> 00:38:22.346 cascade that typically will result in

NOTE Confidence: 0.790147268

00:38:22.414 --> 00:38:24.586 the production of type 1 interferons

NOTE Confidence: 0.790147268

00:38:24.586 --> 00:38:27.280 which of course a very potent

NOTE Confidence: 0.790147268

00:38:27.280 --> 00:38:30.226 antiviral and anti tumor activity.

NOTE Confidence: 0.790147268

00:38:30.226 --> 00:38:33.488 Now this is only true when you

NOTE Confidence: 0.790147268

00:38:33.488 --> 00:38:36.360 activate the pathway very briefly,

NOTE Confidence: 0.790147268

00:38:36.360 --> 00:38:37.800 but this is not really what

NOTE Confidence: 0.790147268

00:38:37.800 --> 00:38:39.293 we see in cancer, right?

NOTE Confidence: 0.790147268

00:38:39.293 --> 00:38:41.958 Because chromosomal instability is perpetual,

NOTE Confidence: 0.790147268

00:38:41.960 --> 00:38:44.480 this pathway is tonically activated.

NOTE Confidence: 0.790147268

00:38:44.480 --> 00:38:46.919 And it turns out that when you do that,

NOTE Confidence: 0.790147268

00:38:46.920 --> 00:38:48.072 you actually flip the

NOTE Confidence: 0.790147268

00:38:48.072 --> 00:38:49.512 entire pathway on its head.

NOTE Confidence: 0.790147268

00:38:49.520 --> 00:38:51.968 And and this is what I want to

NOTE Confidence: 0.790147268

00:38:51.968 --> 00:38:53.974 demonstrate in in in the next few slides

NOTE Confidence: 0.790147268

00:38:53.974 --> 00:38:56.705 and how we might be able to use this

NOTE Confidence: 0.790147268

00:38:56.705 --> 00:38:58.560 information to target this process.

NOTE Confidence: 0.790147268

00:38:58.560 --> 00:39:00.919 So when you tonically activate the pathway,

NOTE Confidence: 0.790147268

00:39:00.920 --> 00:39:03.286 you in fact see less type 1

NOTE Confidence: 0.790147268

00:39:03.286 --> 00:39:05.347 inference production and you see a

NOTE Confidence: 0.790147268

00:39:05.347 --> 00:39:06.960 more aggressive phenotype sort of

NOTE Confidence: 0.790147268

00:39:06.960 --> 00:39:08.880 flipping the pathway on its head.

NOTE Confidence: 0.790147268

00:39:08.880 --> 00:39:10.968 So we wanted to test this hypothesis and

NOTE Confidence: 0.790147268

00:39:10.968 --> 00:39:13.278 we did a couple of simple experiments.

NOTE Confidence: 0.790147268

00:39:13.280 --> 00:39:16.616 First, we asked you know are LKB 1

NOTE Confidence: 0.790147268

00:39:16.616 --> 00:39:18.988 deficient cells in fact less capable

NOTE Confidence: 0.790147268

00:39:18.988 --> 00:39:21.158 of activating type 1 interferon

NOTE Confidence: 0.790147268

00:39:21.158 --> 00:39:23.501 related pathways And this is what

NOTE Confidence: 0.790147268

00:39:23.501 --> 00:39:24.797 I'm showing you here.

NOTE Confidence: 0.790147268

00:39:24.800 --> 00:39:26.830 We have these cell lines that we

NOTE Confidence: 0.790147268

00:39:26.830 --> 00:39:28.125 stimulated with double stranded

NOTE Confidence: 0.790147268

00:39:28.125 --> 00:39:30.309 DNA as sort of surrogate for

NOTE Confidence: 0.790147268

00:39:30.309 --> 00:39:31.037 chromosomal instability.

NOTE Confidence: 0.790147268

00:39:31.040 --> 00:39:33.000 And then we looked at a couple

NOTE Confidence: 0.790147268

00:39:33.000 --> 00:39:34.799 of key downstream nodes from in

NOTE Confidence: 0.790147268

00:39:34.799 --> 00:39:35.999 the C gasting pathway,

NOTE Confidence: 0.790147268

00:39:36.000 --> 00:39:38.954 phosphor TBK one and phosphor IR 3.

NOTE Confidence: 0.790147268

00:39:38.960 --> 00:39:41.304 As you can see the LKB 1 proficient

NOTE Confidence: 0.790147268

00:39:41.304 --> 00:39:43.839 Syn low cell lines are able to

NOTE Confidence: 0.790147268

00:39:43.839 --> 00:39:45.714 do that rather efficiently while

NOTE Confidence: 0.790147268

00:39:45.787 --> 00:39:48.034 the LKB 1 deficient lines do not.

NOTE Confidence: 0.790147268

00:39:48.040 --> 00:39:50.025 This results in a significant

NOTE Confidence: 0.790147268

00:39:50.025 --> 00:39:52.496 impairment in the LKB 1 deficient

NOTE Confidence: 0.790147268

00:39:52.496 --> 00:39:54.914 lines with respect to a couple

NOTE Confidence: 0.790147268

00:39:54.914 --> 00:39:56.472 of important Type 1 interference,

NOTE Confidence: 0.790147268

00:39:56.472 --> 00:39:58.920 I'm just showing you a couple selected ones.

NOTE Confidence: 0.790147268

00:39:58.920 --> 00:40:00.484 So suggesting that these,

NOTE Confidence: 0.790147268

00:40:00.484 --> 00:40:03.517 the Syn high state in fact confers

NOTE Confidence: 0.790147268

00:40:03.517 --> 00:40:06.040 impaired production of type 1 interference.

NOTE Confidence: 0.790147268

00:40:06.040 --> 00:40:08.524 So how can we now prove that it is

NOTE Confidence: 0.790147268

00:40:08.524 --> 00:40:10.917 sin that drives this impairment?

NOTE Confidence: 0.790147268

00:40:10.920 --> 00:40:13.504 So one way to do this is by

NOTE Confidence: 0.790147268

00:40:13.504 --> 00:40:15.912 modulating either up or down the

NOTE Confidence: 0.790147268

00:40:15.912 --> 00:40:17.600 rate of chromosomal instability.

NOTE Confidence: 0.790147268

00:40:17.600 --> 00:40:20.606 And one way to do this is by over

NOTE Confidence: 0.790147268

00:40:20.606 --> 00:40:23.059 expressing a variety of different

NOTE Confidence: 0.790147268

00:40:23.059 --> 00:40:25.095 genetic constructs which had

NOTE Confidence: 0.790147268

00:40:25.095 --> 00:40:26.457 been previously established.

NOTE Confidence: 0.790147268

00:40:26.457 --> 00:40:29.490 So in this example we can take a cell

NOTE Confidence: 0.790147268

00:40:29.559 --> 00:40:31.579 line that is highly chromosomally

NOTE Confidence: 0.790147268

00:40:31.579 --> 00:40:34.280 unstable at baseline LKB 1 deficient

NOTE Confidence: 0.790147268

00:40:34.280 --> 00:40:36.555 and over express a gene called MCAC,

NOTE Confidence: 0.790147268

00:40:36.560 --> 00:40:38.780 also known as KF2C,

NOTE Confidence: 0.790147268

00:40:38.780 --> 00:40:41.000 which improves the segregation

NOTE Confidence: 0.790147268

00:40:41.000 --> 00:40:43.372 fidelity that cells have when

NOTE Confidence: 0.790147268

00:40:43.372 --> 00:40:44.836 they undergo A chromosome.

NOTE Confidence: 0.790147268

00:40:44.840 --> 00:40:47.157 Segregation in so many words reduce the

NOTE Confidence: 0.790147268

00:40:47.157 --> 00:40:49.582 number of errors that these cells make

NOTE Confidence: 0.790147268

00:40:49.582 --> 00:40:51.640 and therefore on a population level,

NOTE Confidence: 0.790147268

00:40:51.640 --> 00:40:53.840 the rate of chromosomal instability,

NOTE Confidence: 0.790147268

00:40:53.840 --> 00:40:56.052 which is again measured here as the

NOTE Confidence: 0.790147268

00:40:56.052 --> 00:40:57.920 number of frequency of micronuclei.

NOTE Confidence: 0.790147268

00:40:57.920 --> 00:41:00.836 So when you suppress chromosomal instability,

NOTE Confidence: 0.790147268

00:41:00.840 --> 00:41:03.546 that alone is sufficient to rescue

NOTE Confidence: 0.790147268

00:41:03.546 --> 00:41:06.439 the ability of these cells to

NOTE Confidence: 0.790147268

00:41:06.439 --> 00:41:08.954 again produce type 1 interference.

NOTE Confidence: 0.790147268

00:41:08.960 --> 00:41:10.598 You can do the converse experiment

NOTE Confidence: 0.790147268

00:41:10.598 --> 00:41:13.165 where you take a cell line that is

NOTE Confidence: 0.790147268

00:41:13.165 --> 00:41:14.581 relatively chromosomally stable and

NOTE Confidence: 0.790147268

00:41:14.581 --> 00:41:16.699 express them with a different construct

NOTE Confidence: 0.790147268

00:41:16.699 --> 00:41:18.394 that makes them more unstable,

NOTE Confidence: 0.790147268

00:41:18.400 --> 00:41:21.200 as shown here again as a measure

NOTE Confidence: 0.790147268

00:41:21.200 --> 00:41:22.000 of micronuclei.

NOTE Confidence: 0.790147268

00:41:22.000 --> 00:41:24.527 And that alone is sufficient to reduce

NOTE Confidence: 0.790147268

00:41:24.527 --> 00:41:27.520 their ability to produce type 1 interference,

NOTE Confidence: 0.790147268

00:41:27.520 --> 00:41:29.510 suggesting that it is really

NOTE Confidence: 0.790147268

00:41:29.510 --> 00:41:31.500 sin that's driving the ability

NOTE Confidence: 0.872830699375

00:41:31.573 --> 00:41:33.941 or inability of these cells to properly

NOTE Confidence: 0.872830699375

00:41:33.941 --> 00:41:35.646 signal through this through this

NOTE Confidence: 0.872830699375

00:41:35.646 --> 00:41:37.840 pathway and produce type 1 interference.

NOTE Confidence: 0.872830699375

00:41:37.840 --> 00:41:40.801 The other way to to approach this of course

NOTE Confidence: 0.872830699375

00:41:40.801 --> 00:41:43.961 is to imagine either deleting genetically

NOTE Confidence: 0.872830699375

00:41:43.961 --> 00:41:46.716 or pharmacologically inhibiting C gas.

NOTE Confidence: 0.872830699375

00:41:46.720 --> 00:41:48.070 And the rationale here is

NOTE Confidence: 0.872830699375

00:41:48.070 --> 00:41:49.720 if you don't have C gas,

NOTE Confidence: 0.872830699375

00:41:49.720 --> 00:41:52.786 then you won't be able to tonically

NOTE Confidence: 0.872830699375

00:41:52.786 --> 00:41:55.040 activate the pathway downstream.

NOTE Confidence: 0.872830699375

00:41:55.040 --> 00:41:57.302 And by at least temporarily relieving

NOTE Confidence: 0.872830699375

00:41:57.302 --> 00:41:59.409 the tonic activation through genetic

NOTE Confidence: 0.872830699375

00:41:59.409 --> 00:42:01.557 deletion of pharmacological inhibition,

NOTE Confidence: 0.872830699375

00:42:01.560 --> 00:42:03.810 you might be able to allow this thing to

NOTE Confidence: 0.872830699375

00:42:03.810 --> 00:42:06.568 come back to an equilibrium where you can

NOTE Confidence: 0.872830699375

00:42:06.568 --> 00:42:08.535 leverage its physiological function which

NOTE Confidence: 0.872830699375

00:42:08.535 --> 00:42:10.680 is producing these important cytokines.

NOTE Confidence: 0.872830699375

00:42:10.680 --> 00:42:12.696 So we tested this in a number

NOTE Confidence: 0.872830699375

00:42:12.696 --> 00:42:13.560 of different ways.

NOTE Confidence: 0.872830699375

00:42:13.560 --> 00:42:16.668 One is by deleting Cgas and then

NOTE Confidence: 0.872830699375

00:42:16.668 --> 00:42:19.296 stimulating the cells with the with

NOTE Confidence: 0.872830699375

00:42:19.296 --> 00:42:22.117 its natural product that is CGAM O,

NOTE Confidence: 0.872830699375

00:42:22.120 --> 00:42:22.945 we delete Cgas.

NOTE Confidence: 0.872830699375

00:42:22.945 --> 00:42:25.154 We let the cells sort of relax for

NOTE Confidence: 0.872830699375

00:42:25.154 --> 00:42:27.143 a week or two and then we ask was

NOTE Confidence: 0.872830699375

00:42:27.208 --> 00:42:29.469 that enough to bring the pathway to

NOTE Confidence: 0.872830699375

00:42:29.469 --> 00:42:31.403 an equilibrium and stimulate it and

NOTE Confidence: 0.872830699375

00:42:31.403 --> 00:42:33.329 show that they're now again able

NOTE Confidence: 0.872830699375

00:42:33.329 --> 00:42:35.440 to produce type 1 interference.

NOTE Confidence: 0.872830699375

00:42:35.440 --> 00:42:37.316 And this is precisely what we find.

NOTE Confidence: 0.872830699375

00:42:37.320 --> 00:42:39.904 So after a few days of of of

NOTE Confidence: 0.872830699375

00:42:39.904 --> 00:42:42.080 of of deleting C gas,

NOTE Confidence: 0.872830699375

00:42:42.080 --> 00:42:44.372 genetically stimulating the cells with C

NOTE Confidence: 0.872830699375

00:42:44.372 --> 00:42:46.719 gaps or stimulating sting in this case,

NOTE Confidence: 0.872830699375

00:42:46.720 --> 00:42:48.974 we see that these cells regained their

NOTE Confidence: 0.872830699375

00:42:48.974 --> 00:42:50.959 ability to produce Type 1 inference.

NOTE Confidence: 0.872830699375

00:42:50.960 --> 00:42:53.282 And this is both true in the human as

NOTE Confidence: 0.872830699375

00:42:53.282 --> 00:42:55.758 well as in the mouse models that we use.

NOTE Confidence: 0.872830699375

00:42:55.760 --> 00:42:56.948 And lastly we,

NOTE Confidence: 0.872830699375

00:42:56.948 --> 00:42:59.720 we tested these concepts also in vivo.

NOTE Confidence: 0.872830699375

00:42:59.720 --> 00:43:01.760 So here we use the KL,

NOTE Confidence: 0.872830699375

00:43:01.760 --> 00:43:04.610 the LKB 1 deficient line and

NOTE Confidence: 0.872830699375

00:43:04.610 --> 00:43:06.790 implanted them into B6 mice.

NOTE Confidence: 0.872830699375

00:43:06.790 --> 00:43:08.665 We treated these animals either

NOTE Confidence: 0.872830699375

00:43:08.665 --> 00:43:10.835 with with isotype control or with

NOTE Confidence: 0.872830699375

00:43:10.835 --> 00:43:13.103 an anti PD1 antibody and just like

NOTE Confidence: 0.872830699375

00:43:13.167 --> 00:43:15.142 in patients there is absolutely

NOTE Confidence: 0.872830699375

00:43:15.142 --> 00:43:17.651 no response to PD1 inhibition and

NOTE Confidence: 0.872830699375

00:43:17.651 --> 00:43:20.837 these LKB 1 deficient Synthi tumors.

NOTE Confidence: 0.872830699375

00:43:20.840 --> 00:43:23.280 Deleting Cgas alone was sufficient

NOTE Confidence: 0.872830699375

00:43:23.280 --> 00:43:26.775 to partly reduce the growth rate of

NOTE Confidence: 0.872830699375

00:43:26.775 --> 00:43:29.215 these tumors but also significantly

NOTE Confidence: 0.872830699375

00:43:29.215 --> 00:43:31.740 sensitized them to PD1 inhibition.

NOTE Confidence: 0.872830699375

00:43:31.740 --> 00:43:34.920 The converse experiment we also did

NOTE Confidence: 0.872830699375

00:43:34.920 --> 00:43:37.510 is taking the KP cell line which

NOTE Confidence: 0.872830699375

00:43:37.510 --> 00:43:39.282 are relatively sensitive to PD1

NOTE Confidence: 0.872830699375

00:43:39.282 --> 00:43:41.368 inhibition as you can see here and

NOTE Confidence: 0.872830699375

00:43:41.368 --> 00:43:43.877 make them more chromosomally unstable.

NOTE Confidence: 0.872830699375

00:43:43.880 --> 00:43:46.295 And this time we show that sin

NOTE Confidence: 0.872830699375

00:43:46.295 --> 00:43:48.859 elevating sin alone is sufficient to

NOTE Confidence: 0.872830699375

00:43:48.859 --> 00:43:51.279 render them resistant to immunotherapy.

NOTE Confidence: 0.872830699375

00:43:51.280 --> 00:43:54.094 This is work that we have done

NOTE Confidence: 0.872830699375

00:43:54.094 --> 00:43:56.159 in collaboration with Sam Bakum,

NOTE Confidence: 0.872830699375

00:43:56.160 --> 00:43:58.548 the other Bakum and and Chrissy

NOTE Confidence: 0.872830699375

00:43:58.548 --> 00:44:00.879 Hong a postdoc in his lap.

NOTE Confidence: 0.889359795833334

00:44:02.920 --> 00:44:05.258 Of course we're now interested in moving

NOTE Confidence: 0.889359795833334

00:44:05.258 --> 00:44:06.960 these concepts closer to patients.

NOTE Confidence: 0.889359795833334

00:44:06.960 --> 00:44:08.660 The problem is that there

NOTE Confidence: 0.889359795833334

00:44:08.660 --> 00:44:10.742 is no known, you know,

NOTE Confidence: 0.889359795833334

00:44:10.742 --> 00:44:13.397 soluble human selective CS inhibitor.

NOTE Confidence: 0.889359795833334

00:44:13.400 --> 00:44:16.284 So we work with our medicinal chemists

NOTE Confidence: 0.889359795833334

00:44:16.284 --> 00:44:18.880 to actually develop such an inhibitor.

NOTE Confidence: 0.889359795833334

00:44:18.880 --> 00:44:21.688 And what I'm showing you here is the REDUCT

NOTE Confidence: 0.889359795833334

00:44:21.688 --> 00:44:23.680 redacted structure of that compound.

NOTE Confidence: 0.889359795833334

00:44:23.680 --> 00:44:24.712 And on the bottom,

NOTE Confidence: 0.889359795833334

00:44:24.712 --> 00:44:26.616 the functional assay that we use to

NOTE Confidence: 0.889359795833334

00:44:26.616 --> 00:44:28.434 determine the activity of the compound.

NOTE Confidence: 0.889359795833334

00:44:28.440 --> 00:44:30.066 We're looking at the levels of

NOTE Confidence: 0.889359795833334

00:44:30.066 --> 00:44:31.709 CGAMP where we treated the cell

NOTE Confidence: 0.889359795833334

00:44:31.709 --> 00:44:33.466 lines that are we've you know I've

NOTE Confidence: 0.889359795833334

00:44:33.466 --> 00:44:35.315 shown you throughout this talk with

NOTE Confidence: 0.889359795833334

00:44:35.315 --> 00:44:37.520 this with this new compound and we

NOTE Confidence: 0.889359795833334

00:44:37.520 --> 00:44:39.644 see that we can pretty potently

NOTE Confidence: 0.889359795833334

00:44:39.644 --> 00:44:42.162 suppress the production of of C gas

NOTE Confidence: 0.889359795833334

00:44:42.162 --> 00:44:44.076 meaning the activity of C gas.

NOTE Confidence: 0.889359795833334

00:44:44.080 --> 00:44:46.396 And just treating the cells with

NOTE Confidence: 0.889359795833334

00:44:46.396 --> 00:44:49.519 these with the C gas inhibitor alone

NOTE Confidence: 0.889359795833334

00:44:49.520 --> 00:44:52.118 results in reconstitution of these set

NOTE Confidence: 0.889359795833334

00:44:52.118 --> 00:44:54.791 of these cell lines to phosphorylate

NOTE Confidence: 0.889359795833334

00:44:54.791 --> 00:44:57.822 TBK one and IR three more efficiently

NOTE Confidence: 0.889359795833334

00:44:57.822 --> 00:45:00.716 and ultimately result in an improved

NOTE Confidence: 0.889359795833334

00:45:00.720 --> 00:45:04.758 ability to produce these important cytokines.

NOTE Confidence: 0.889359795833334

00:45:04.760 --> 00:45:07.000 So with that, I want to summarize

NOTE Confidence: 0.889359795833334

00:45:07.000 --> 00:45:08.759 this last part of my talk.

NOTE Confidence: 0.889359795833334

00:45:08.760 --> 00:45:10.840 Hope I was able to show you that,

NOTE Confidence: 0.889359795833334

00:45:10.840 --> 00:45:12.565 you know not all metastases

NOTE Confidence: 0.889359795833334

00:45:12.565 --> 00:45:13.600 are created equal.

NOTE Confidence: 0.889359795833334

00:45:13.600 --> 00:45:16.516 Brain meds are quite distinct genomically.

NOTE Confidence: 0.889359795833334

00:45:16.520 --> 00:45:19.880 That tonic activation of the C gas

NOTE Confidence: 0.889359795833334

00:45:19.880 --> 00:45:22.585 thing pathway through sin is is bad

NOTE Confidence: 0.889359795833334

00:45:22.585 --> 00:45:24.858 and results in suppression of Type

NOTE Confidence: 0.889359795833334

00:45:24.858 --> 00:45:27.570 1 interference that can be rescued

NOTE Confidence: 0.889359795833334

00:45:27.570 --> 00:45:29.640 through genetic modulation of sin

NOTE Confidence: 0.889359795833334

00:45:29.640 --> 00:45:31.880 or inhibition or deletion of C gas.

NOTE Confidence: 0.889359795833334

00:45:31.880 --> 00:45:33.236 And that we are, you know,

NOTE Confidence: 0.889359795833334

00:45:33.240 --> 00:45:36.856 very interested in moving this into the

NOTE Confidence: 0.889359795833334

00:45:36.856 --> 00:45:39.452 clinic using AC gas inhibitor of course.

NOTE Confidence: 0.889359795833334

00:45:39.452 --> 00:45:40.104 And I,

NOTE Confidence: 0.889359795833334

00:45:40.104 --> 00:45:40.756 you know,

NOTE Confidence: 0.889359795833334

00:45:40.760 --> 00:45:42.485 mentioned the people who have

NOTE Confidence: 0.889359795833334

00:45:42.485 --> 00:45:43.800 done the work throughout,

NOTE Confidence: 0.889359795833334

00:45:43.800 --> 00:45:45.480 but this is their entire lab.

NOTE Confidence: 0.889359795833334

00:45:45.480 --> 00:45:47.209 I want to think and of course

NOTE Confidence: 0.889359795833334

00:45:47.209 --> 00:45:48.880 all of the collaborators,

NOTE Confidence: 0.889359795833334

00:45:48.880 --> 00:45:50.304 both nationally and international

NOTE Confidence: 0.889359795833334

00:45:50.304 --> 00:45:52.791 collaborators who we are lucky to work

NOTE Confidence: 0.889359795833334

00:45:52.791 --> 00:45:54.870 with and the funding sources that support

NOTE Confidence: 0.889359795833334

00:45:54.870 --> 00:45:56.800 this work happy to take questions.

NOTE Confidence: 0.95533897

00:46:06.320 --> 00:46:08.035 Thank you Ben for a wonderful talk.

NOTE Confidence: 0.93598824625

00:46:08.040 --> 00:46:10.880 I think you're very deserving of the plaque.

NOTE Confidence: 0.93598824625

00:46:10.880 --> 00:46:12.440 While people think what they want to ask.

NOTE Confidence: 0.93598824625

00:46:12.440 --> 00:46:13.700 I'm going to ask you a question

NOTE Confidence: 0.93598824625

00:46:13.700 --> 00:46:16.320 about the CD2 CD 58 access.

NOTE Confidence: 0.819053892

00:46:16.880 --> 00:46:19.240 If you up regulate CD2,

NOTE Confidence: 0.819053892

00:46:19.240 --> 00:46:20.878 do you get reciprocal up regulation

NOTE Confidence: 0.819053892

00:46:20.880 --> 00:46:22.230 of CD 58 because that might

NOTE Confidence: 0.819053892

00:46:22.230 --> 00:46:23.793 be another way to approach.

NOTE Confidence: 0.819053892

00:46:23.793 --> 00:46:25.890 If you up regulate CD2 because

NOTE Confidence: 0.819053892

00:46:25.890 --> 00:46:27.720 you were focused on the tumor

NOTE Confidence: 0.811676233076923

00:46:27.720 --> 00:46:29.449 cell with CD 58, but can you

NOTE Confidence: 0.811676233076923

00:46:29.449 --> 00:46:30.839 manipulate it via the T cell?

NOTE Confidence: 0.89907039

00:46:30.920 --> 00:46:33.600 Yes. So we we did the other way around also.

NOTE Confidence: 0.89907039

00:46:33.600 --> 00:46:36.640 So we when we I guess I should talk here.

NOTE Confidence: 0.89907039

00:46:36.640 --> 00:46:38.572 So so we did the other way

NOTE Confidence: 0.89907039

00:46:38.572 --> 00:46:40.660 around where we knocked out CD2

NOTE Confidence: 0.89907039

00:46:40.660 --> 00:46:43.880 and rescued it in the T cells.

NOTE Confidence: 0.89907039

00:46:43.880 --> 00:46:46.421 The interesting part there is of course

NOTE Confidence: 0.89907039

00:46:46.421 --> 00:46:49.623 deletion of CD2 is basically leads to non

NOTE Confidence: 0.89907039

00:46:49.623 --> 00:46:52.200 responsiveness irrespective of CD 58 status.

NOTE Confidence: 0.89907039

00:46:52.200 --> 00:46:54.342 But the interesting observation and and and

NOTE Confidence: 0.89907039

00:46:54.342 --> 00:46:56.923 that might be an artifact of the system is

NOTE Confidence: 0.89907039

00:46:56.923 --> 00:47:00.384 when you over express CD2 through an ORPH,

NOTE Confidence: 0.89907039

00:47:00.384 --> 00:47:04.612 then you see actually suppression of CD 58.

NOTE Confidence: 0.89907039

00:47:04.612 --> 00:47:06.330 So we can't just change the

NOTE Confidence: 0.89907039

00:47:06.330 --> 00:47:07.560 T cell in that case. Yeah.

NOTE Confidence: 0.757696043333333

00:47:10.240 --> 00:47:11.278 All right. Questions.

NOTE Confidence: 0.757696043333333

00:47:11.280 --> 00:47:12.240 And I'm sure there's some

NOTE Confidence: 0.757696043333333

00:47:12.240 --> 00:47:13.800 online there 50 something people

NOTE Confidence: 0.660784666

00:47:13.800 --> 00:47:16.040 online I saw some have left bones. I didn't

NOTE Confidence: 0.64162793

00:47:17.880 --> 00:47:19.200 think the microphones working.

NOTE Confidence: 0.410130966666667

00:47:20.960 --> 00:47:21.599 OK Chen. Yeah.

NOTE Confidence: 0.5593943

00:47:24.240 --> 00:47:26.480 I guess I can click the. Yes,

NOTE Confidence: 0.6048490025

00:47:26.480 --> 00:47:28.640 I can click the very interesting

NOTE Confidence: 0.751295871428571

00:47:28.640 --> 00:47:32.480 work. I have a question about the third part.

NOTE Confidence: 0.751295871428571

00:47:32.480 --> 00:47:35.000 You have to show those result

NOTE Confidence: 0.751295871428571

00:47:35.000 --> 00:47:36.680 in primary tumor setting.

NOTE Confidence: 0.751295871428571

00:47:36.680 --> 00:47:38.684 Have you looked at in metastas

NOTE Confidence: 0.751295871428571

00:47:38.684 --> 00:47:40.560 setting in brain metastas setting?

NOTE Confidence: 0.950270709

00:47:41.960 --> 00:47:43.560 No, because we don't have

NOTE Confidence: 0.950270709

00:47:43.560 --> 00:47:45.160 the right model for it.

NOTE Confidence: 0.950270709

00:47:45.160 --> 00:47:48.800 And I think that everyone who works on

NOTE Confidence: 0.950270709

00:47:48.800 --> 00:47:50.800 trying to understand brain metastasis,

NOTE Confidence: 0.950270709

00:47:50.800 --> 00:47:52.696 this was sort of how we

NOTE Confidence: 0.950270709

00:47:52.696 --> 00:47:53.960 got interested in this.

NOTE Confidence: 0.950270709

00:47:53.960 --> 00:47:55.946 One of the challenges is that

NOTE Confidence: 0.950270709

00:47:55.946 --> 00:47:57.760 to my knowledge at least,

NOTE Confidence: 0.950270709

00:47:57.760 --> 00:48:00.825 there isn't a good immunocompetent

NOTE Confidence: 0.950270709

00:48:00.825 --> 00:48:03.295 model that has a sufficiently high

NOTE Confidence: 0.950270709

00:48:03.295 --> 00:48:05.150 rate of developing brain metastasis

NOTE Confidence: 0.950270709

00:48:05.217 --> 00:48:06.957 in a number of different ways,

NOTE Confidence: 0.950270709

00:48:06.960 --> 00:48:08.916 at least through sort of natural

NOTE Confidence: 0.950270709

00:48:08.920 --> 00:48:10.180 sort of metastatic routes.

NOTE Confidence: 0.950270709

00:48:10.180 --> 00:48:12.070 So we haven't been able to

NOTE Confidence: 0.950270709

00:48:12.133 --> 00:48:14.063 actually directly study this in

NOTE Confidence: 0.950270709

00:48:14.063 --> 00:48:15.993 brain metastasis for that reason.

NOTE Confidence: 0.855275058333333

00:48:16.560 --> 00:48:19.073 So I assume some of the young

NOTE Confidence: 0.855275058333333

00:48:19.073 --> 00:48:21.038 models are LKB. One loss,

NOTE Confidence: 0.855275058333333

00:48:21.040 --> 00:48:23.077 maybe some of those could be positive,

NOTE Confidence: 0.66934526

00:48:26.400 --> 00:48:26.600 Yeah,

NOTE Confidence: 0.818434204

00:48:30.320 --> 00:48:33.696 yeah. But even putting the KL the the

NOTE Confidence: 0.818434204

00:48:33.696 --> 00:48:37.277 mouse cell lines that I've shown earlier,

NOTE Confidence: 0.818434204

00:48:37.280 --> 00:48:39.863 even if you do an LV injection

NOTE Confidence: 0.818434204

00:48:39.863 --> 00:48:41.800 or an intracarotic injection,

NOTE Confidence: 0.818434204

00:48:41.800 --> 00:48:44.670 they don't see the brain for some

NOTE Confidence: 0.818434204

00:48:44.670 --> 00:48:47.999 reason which we don't really understand.

NOTE Confidence: 0.818434204

00:48:48.000 --> 00:48:49.398 But what was the second part?

NOTE Confidence: 0.818434204

00:48:49.400 --> 00:48:51.878 You had another part to your question?

NOTE Confidence: 0.818434204

00:48:51.880 --> 00:48:53.240 That's yes.

NOTE Confidence: 0.818434204

00:48:53.240 --> 00:48:55.760 So, so I think it's just a limitation

NOTE Confidence: 0.818434204

00:48:55.760 --> 00:48:58.678 right now in terms of the available models.

NOTE Confidence: 0.818434204

00:48:58.680 --> 00:48:58.920 Since

NOTE Confidence: 0.635713536

00:48:58.920 --> 00:49:00.860 I'm sitting next to Chen, I'll take

NOTE Confidence: 0.635713536

00:49:00.860 --> 00:49:02.720 advantage to ask a quick question.

NOTE Confidence: 0.86694576

00:49:03.520 --> 00:49:05.998 So the CMTM 6 in the first part

NOTE Confidence: 0.86694576

00:49:05.998 --> 00:49:07.560 of the talk is interesting,

NOTE Confidence: 0.834580795833333

00:49:07.560 --> 00:49:09.604 but the binding region that you identified

NOTE Confidence: 0.834580795833333

00:49:09.604 --> 00:49:11.719 is in the extracellular domain.

NOTE Confidence: 0.834580795833333

00:49:11.720 --> 00:49:13.040 I thought it was a stabilizer

NOTE Confidence: 0.834580795833333

00:49:13.040 --> 00:49:14.705 binding to the cytoplasmic domain

NOTE Confidence: 0.834580795833333

00:49:14.705 --> 00:49:16.240 and the transmembrane protein, but

NOTE Confidence: 0.895742608

00:49:16.440 --> 00:49:18.225 it's actually it's actually the

NOTE Confidence: 0.895742608

00:49:18.225 --> 00:49:20.456 extracellular domain and it has two

NOTE Confidence: 0.895742608

00:49:20.456 --> 00:49:22.628 extracellular domains which we we we

NOTE Confidence: 0.895742608

00:49:22.628 --> 00:49:25.140 we also mutated and and shown in the

NOTE Confidence: 0.895742608

00:49:25.218 --> 00:49:27.463 paper that both extracellular domains

NOTE Confidence: 0.895742608

00:49:27.463 --> 00:49:30.622 of CMTM 6 are required for stabilizing

NOTE Confidence: 0.895742608

00:49:30.622 --> 00:49:33.280 both PDL one as well as CD 58.

NOTE Confidence: 0.895742608

00:49:33.280 --> 00:49:34.810 So it's clearly the extracellular

NOTE Confidence: 0.895742608

00:49:34.810 --> 00:49:36.034 domains that are necessary.

NOTE Confidence: 0.756327499090909

00:49:36.480 --> 00:49:37.800 It's a stabilization phenomenon

NOTE Confidence: 0.756327499090909

00:49:37.800 --> 00:49:39.960 that is a function of CMTM 6.

NOTE Confidence: 0.879085343333333

00:49:39.960 --> 00:49:41.520 So the function of CMTM 6,

NOTE Confidence: 0.879085343333333

00:49:41.520 --> 00:49:43.788 I didn't get to the into this

NOTE Confidence: 0.879085343333333

00:49:43.788 --> 00:49:46.159 in detail is it's it shuttles,

NOTE Confidence: 0.879085343333333

00:49:46.160 --> 00:49:48.845 it shuttles its cargo through

NOTE Confidence: 0.879085343333333

00:49:48.845 --> 00:49:50.993 through the recycling endosome.

NOTE Confidence: 0.879085343333333

00:49:51.000 --> 00:49:53.424 So typically when proteins are bound

NOTE Confidence: 0.879085343333333

00:49:53.424 --> 00:49:56.400 to CMTM 6, they are shuttled back and

NOTE Confidence: 0.879085343333333

00:49:56.400 --> 00:49:58.840 recycled back to the cell membrane.

NOTE Confidence: 0.879085343333333

00:49:58.840 --> 00:50:00.640 When you lose, I'm shown this in the paper.

NOTE Confidence: 0.879085343333333

00:50:00.640 --> 00:50:02.320 When you delete CMTM 6,

NOTE Confidence: 0.879085343333333

00:50:02.320 --> 00:50:04.504 you can show that they're more

NOTE Confidence: 0.879085343333333

00:50:04.504 --> 00:50:05.596 preferentially lysosomally degraded.

NOTE Confidence: 0.808377375

00:50:19.070 --> 00:50:21.668 Oh, thank you. Very fantastic talk.

NOTE Confidence: 0.808377375

00:50:21.670 --> 00:50:23.650 I'm extremely interested in the

NOTE Confidence: 0.808377375

00:50:23.650 --> 00:50:26.080 the third part of your talk,

NOTE Confidence: 0.808377375

00:50:26.080 --> 00:50:29.816 the LKP one seems like it's leading to

NOTE Confidence: 0.808377375

00:50:29.816 --> 00:50:32.376 leads to the chromosome's instability.

NOTE Confidence: 0.808377375

00:50:32.376 --> 00:50:35.542 But however in my impression now

NOTE Confidence: 0.808377375

00:50:35.542 --> 00:50:37.230 we think chromosome instability

NOTE Confidence: 0.808377375

00:50:37.230 --> 00:50:39.880 leads to more like mutation burden,

NOTE Confidence: 0.808377375

00:50:39.880 --> 00:50:42.931 which is a good thing for you know

NOTE Confidence: 0.808377375

00:50:42.931 --> 00:50:44.919 response to to immunotherapy.

NOTE Confidence: 0.808377375

00:50:44.920 --> 00:50:47.468 But in your story seems like it's

NOTE Confidence: 0.808377375

00:50:47.468 --> 00:50:50.576 on the other way and also you know

NOTE Confidence: 0.808377375

00:50:50.576 --> 00:50:53.486 some point that we know the PD1

NOTE Confidence: 0.808377375

00:50:53.486 --> 00:50:55.316 blocking antibody is approved for

NOTE Confidence: 0.808377375

00:50:55.316 --> 00:50:57.772 all cancer which has the MSI high,

NOTE Confidence: 0.808377375

00:50:57.772 --> 00:50:59.236 you know your tumors.

NOTE Confidence: 0.808377375

00:50:59.240 --> 00:51:01.074 So I wonder if you have any

NOTE Confidence: 0.808377375

00:51:01.074 --> 00:51:02.200 comments between on this.

NOTE Confidence: 0.808377375

00:51:02.200 --> 00:51:06.625 And also my second question is when you I,

NOTE Confidence: 0.808377375

00:51:06.625 --> 00:51:09.235 I know you're planning to put

NOTE Confidence: 0.808377375

00:51:09.235 --> 00:51:12.277 the C gas inhibitor on clinic,

NOTE Confidence: 0.808377375

00:51:12.280 --> 00:51:13.056 what's your,

NOTE Confidence: 0.808377375

00:51:13.056 --> 00:51:15.384 what's the approach in your mind

NOTE Confidence: 0.808377375

00:51:15.384 --> 00:51:18.015 because now we know there's a lot

NOTE Confidence: 0.808377375

00:51:18.015 --> 00:51:19.815 of steam possibly agonist those

NOTE Confidence: 0.808377375

00:51:19.815 --> 00:51:22.440 of it has been tested in clinic.

NOTE Confidence: 0.808377375

00:51:22.440 --> 00:51:24.520 So seems like you know,

NOTE Confidence: 0.808377375

00:51:24.520 --> 00:51:27.930 I I don't know if you ever discussed with

NOTE Confidence: 0.808377375

00:51:27.930 --> 00:51:33.400 Thomas Kiosky because he's a you know,

NOTE Confidence: 0.808377375

00:51:33.400 --> 00:51:35.278 yeah thing guy.

NOTE Confidence: 0.808377375

00:51:35.280 --> 00:51:35.720 So

NOTE Confidence: 0.874233872352941

00:51:35.720 --> 00:51:37.970 I am very happy that you asked both of

NOTE Confidence: 0.874233872352941

00:51:37.970 --> 00:51:40.193 these questions because I think it's

NOTE Confidence: 0.874233872352941

00:51:40.193 --> 00:51:42.024 really important to clarify a couple

NOTE Confidence: 0.874233872352941

00:51:42.024 --> 00:51:44.199 of things and maybe I went too fast.

NOTE Confidence: 0.874233872352941

00:51:44.200 --> 00:51:47.150 So to the first point, yes,

NOTE Confidence: 0.874233872352941

00:51:47.150 --> 00:51:51.000 TMB to some extent is associated weekly,

NOTE Confidence: 0.874233872352941

00:51:51.000 --> 00:51:52.960 but it is associated with

NOTE Confidence: 0.874233872352941

00:51:52.960 --> 00:51:54.844 response to checkpoint inhibition.

NOTE Confidence: 0.874233872352941

00:51:54.844 --> 00:51:58.559 What I was talking about here is not TMB,

NOTE Confidence: 0.874233872352941

00:51:58.560 --> 00:52:00.582 it's not, you know the number

NOTE Confidence: 0.874233872352941

00:52:00.582 --> 00:52:01.593 of non synonymous,

NOTE Confidence: 0.874233872352941

00:52:01.600 --> 00:52:03.838 you know mutations throughout the genome.

NOTE Confidence: 0.874233872352941

00:52:03.840 --> 00:52:05.958 When we talked about chromosomal instability,

NOTE Confidence: 0.874233872352941

00:52:05.960 --> 00:52:09.558 a different form of genome instability that

NOTE Confidence: 0.874233872352941

00:52:09.558 --> 00:52:12.303 is rather characterized by large changes,

NOTE Confidence: 0.874233872352941

00:52:12.303 --> 00:52:13.812 large structural changes,

NOTE Confidence: 0.874233872352941

00:52:13.812 --> 00:52:16.830 loss of chromosome arms or gains

NOTE Confidence: 0.874233872352941

00:52:16.908 --> 00:52:18.890 of entire chromosomes, etc.

NOTE Confidence: 0.874233872352941

00:52:18.890 --> 00:52:21.200 What we were actually able to show,

NOTE Confidence: 0.874233872352941

00:52:21.200 --> 00:52:23.024 and that was on the slide but I

NOTE Confidence: 0.874233872352941

00:52:23.024 --> 00:52:24.878 went over it probably too quickly,

NOTE Confidence: 0.874233872352941

00:52:24.880 --> 00:52:28.075 is that in this particular case of LKB 1,

NOTE Confidence: 0.874233872352941

00:52:28.080 --> 00:52:30.240 the mutation is not associated

NOTE Confidence: 0.874233872352941

00:52:30.240 --> 00:52:32.400 with a difference in TMB.

NOTE Confidence: 0.874233872352941

00:52:32.400 --> 00:52:34.500 It's only associated with a difference

NOTE Confidence: 0.874233872352941

00:52:34.500 --> 00:52:37.560 in the rate of chromosomal instability,

NOTE Confidence: 0.874233872352941

00:52:37.560 --> 00:52:39.378 at least the the genetic surrogate

NOTE Confidence: 0.874233872352941

00:52:39.378 --> 00:52:41.719 of it in in the PCGA cohort.

NOTE Confidence: 0.874233872352941

00:52:41.720 --> 00:52:44.168 So I think these are two different forms

NOTE Confidence: 0.874233872352941

00:52:44.168 --> 00:52:46.438 of genome instability that are very,

NOTE Confidence: 0.874233872352941

00:52:46.440 --> 00:52:48.720 very different. OK.

NOTE Confidence: 0.874233872352941

00:52:48.720 --> 00:52:51.953 And then the second part is you

NOTE Confidence: 0.874233872352941

00:52:51.953 --> 00:52:54.918 know with respect to sting,

NOTE Confidence: 0.874233872352941

00:52:54.920 --> 00:52:56.520 those structures don't work so

NOTE Confidence: 0.874233872352941

00:52:56.520 --> 00:52:57.808 well in patients, right?

NOTE Confidence: 0.874233872352941

00:52:57.808 --> 00:52:58.792 That's the reality,

NOTE Confidence: 0.874233872352941

00:52:58.792 --> 00:52:59.120 right.

NOTE Confidence: 0.874233872352941

00:52:59.120 --> 00:53:01.664 The response rate to a sting

NOTE Confidence: 0.874233872352941

00:53:01.664 --> 00:53:03.360 agonist is relatively low.

NOTE Confidence: 0.874233872352941

00:53:03.360 --> 00:53:06.600 There are and I I had some of them myself,

NOTE Confidence: 0.874233872352941

00:53:06.600 --> 00:53:09.288 there are some patients who are

NOTE Confidence: 0.874233872352941

00:53:09.288 --> 00:53:11.080 exquisitely responsive to them,

NOTE Confidence: 0.874233872352941

00:53:11.080 --> 00:53:13.920 but the vast majority of patients do not.

NOTE Confidence: 0.874233872352941

00:53:13.920 --> 00:53:17.056 And I think that the work that I

NOTE Confidence: 0.874233872352941

00:53:17.056 --> 00:53:19.855 presented here actually supports why that

NOTE Confidence: 0.874233872352941

00:53:19.855 --> 00:53:23.142 might be right because when you have

NOTE Confidence: 0.874233872352941

00:53:23.142 --> 00:53:25.434 this tonic activation of the pathway,

NOTE Confidence: 0.874233872352941

00:53:25.440 --> 00:53:26.040 as I said,

NOTE Confidence: 0.874233872352941

00:53:26.040 --> 00:53:27.440 you sort of you flip the pathway

NOTE Confidence: 0.874233872352941

00:53:27.493 --> 00:53:28.237 bit on its head.

NOTE Confidence: 0.874233872352941

00:53:28.240 --> 00:53:30.840 And so if you throw a sting agonist on there,

NOTE Confidence: 0.874233872352941

00:53:30.840 --> 00:53:32.394 you know it probably doesn't do

NOTE Confidence: 0.874233872352941

00:53:32.394 --> 00:53:34.207 very much right to the at least

NOTE Confidence: 0.874233872352941

00:53:34.207 --> 00:53:35.641 to the cancer cell and perhaps

NOTE Confidence: 0.874233872352941

00:53:35.641 --> 00:53:37.438 it might make the problem worse,

NOTE Confidence: 0.874233872352941

00:53:37.440 --> 00:53:40.240 right if if you stimulating the wrong clade,

NOTE Confidence: 0.874233872352941

00:53:40.240 --> 00:53:42.879 if you will of the downstream signaling.

NOTE Confidence: 0.874233872352941

00:53:42.880 --> 00:53:44.800 What I'm suggesting is that we

NOTE Confidence: 0.874233872352941

00:53:44.800 --> 00:53:46.932 might be able to, you know,

NOTE Confidence: 0.874233872352941

00:53:46.932 --> 00:53:49.446 rescue some of these in agonists,

NOTE Confidence: 0.874233872352941

00:53:49.446 --> 00:53:51.864 but they need to be combined

NOTE Confidence: 0.874233872352941

00:53:51.864 --> 00:53:53.680 in the proper way.

NOTE Confidence: 0.874233872352941

00:53:53.680 --> 00:53:56.200 So you could imagine that if you,

NOTE Confidence: 0.874233872352941

00:53:56.200 --> 00:53:58.272 you know, inhibit sea gas right in

NOTE Confidence: 0.874233872352941

00:53:58.272 --> 00:54:01.117 a in a cell line or in a patient,

NOTE Confidence: 0.874233872352941

00:54:01.120 --> 00:54:01.936 if you will,

NOTE Confidence: 0.874233872352941

00:54:01.936 --> 00:54:03.840 then you allow this pathway to come

NOTE Confidence: 0.874233872352941

00:54:03.897 --> 00:54:05.738 back to an equilibrium, you know,

NOTE Confidence: 0.874233872352941

00:54:05.738 --> 00:54:07.621 and then coming in with the sting

NOTE Confidence: 0.874233872352941

00:54:07.621 --> 00:54:09.158 agonist might be more fruitful.

NOTE Confidence: 0.874233872352941

00:54:09.160 --> 00:54:11.182 So I don't think these are

NOTE Confidence: 0.874233872352941

00:54:11.182 --> 00:54:12.193 mutually exclusive strategies,

NOTE Confidence: 0.874233872352941

00:54:12.200 --> 00:54:13.104 but I think that,

NOTE Confidence: 0.874233872352941

00:54:13.104 --> 00:54:13.556 you know,

NOTE Confidence: 0.874233872352941

00:54:13.560 --> 00:54:15.330 the work suggests that just

NOTE Confidence: 0.874233872352941

00:54:15.330 --> 00:54:17.100 throwing sting agonists on things

NOTE Confidence: 0.874233872352941

00:54:17.167 --> 00:54:19.400 will is unlikely to be beneficial.

NOTE Confidence: 0.490145155

00:54:28.240 --> 00:54:31.320 David, differential output.

NOTE Confidence: 0.330621972

00:54:34.120 --> 00:54:35.760 How's my treatment quoted? How's

NOTE Confidence: 0.47446849

00:54:35.760 --> 00:54:39.040 the self burgering out? Atomic CS activity.

NOTE Confidence: 0.6040632

00:54:45.120 --> 00:54:46.320 Fortunately, somebody figured

NOTE Confidence: 0.6040632

00:54:46.320 --> 00:54:48.120 that out. It wasn't me,

NOTE Confidence: 0.742325126

00:54:48.120 --> 00:54:49.920 but it was Sam's lab.

NOTE Confidence: 0.742325126

00:54:49.920 --> 00:54:51.960 They published a a paper last

NOTE Confidence: 0.742325126

00:54:51.960 --> 00:54:54.280 year in Nature that where they

NOTE Confidence: 0.742325126

00:54:54.280 --> 00:54:57.840 show that tonic activity of sting

NOTE Confidence: 0.742325126

00:54:57.840 --> 00:55:01.120 preferentially induces ER stress.

NOTE Confidence: 0.742325126

00:55:01.120 --> 00:55:03.290 And through ER stress you

NOTE Confidence: 0.742325126

00:55:03.290 --> 00:55:05.800 basically you know a tonic ER,

NOTE Confidence: 0.742325126

00:55:05.800 --> 00:55:08.200 ER stress in this case resulted in the,

NOTE Confidence: 0.742325126

00:55:08.200 --> 00:55:10.200 in the, you know,

NOTE Confidence: 0.742325126

00:55:10.200 --> 00:55:11.200 chromatostatic clade.

NOTE Confidence: 0.742325126

00:55:11.200 --> 00:55:12.560 And they've shown beautifully

NOTE Confidence: 0.742325126

00:55:12.560 --> 00:55:13.920 in in vitro models,

NOTE Confidence: 0.742325126

00:55:13.920 --> 00:55:15.726 in fibroblast models that it doesn't

NOTE Confidence: 0.742325126

00:55:15.726 --> 00:55:17.574 take very much of tonic activation

NOTE Confidence: 0.742325126

00:55:17.574 --> 00:55:19.604 to get to that point where you

NOTE Confidence: 0.742325126

00:55:19.604 --> 00:55:21.565 know if you stimulate once you get

NOTE Confidence: 0.742325126

00:55:21.565 --> 00:55:23.276 a really nice Type 1 interferon.

NOTE Confidence: 0.742325126

00:55:23.276 --> 00:55:25.184 But even after two or three

NOTE Confidence: 0.742325126

00:55:25.184 --> 00:55:26.855 stimulations over a couple of days

NOTE Confidence: 0.742325126

00:55:26.855 --> 00:55:28.640 you very quickly sort of you know,

NOTE Confidence: 0.742325126

00:55:28.640 --> 00:55:30.747 tacky for LAX on that pathway and

NOTE Confidence: 0.742325126

00:55:30.747 --> 00:55:32.526 you start activating the the bad

NOTE Confidence: 0.742325126

00:55:32.526 --> 00:55:34.164 counterpart which is in this case

NOTE Confidence: 0.742325126

00:55:34.164 --> 00:55:37.096 the chronic ER stress activity.

NOTE Confidence: 0.742325126

00:55:37.096 --> 00:55:37.760 OK.

NOTE Confidence: 0.742325126

00:55:37.760 --> 00:55:38.798 I'm going to give the last

NOTE Confidence: 0.742325126

00:55:38.798 --> 00:55:41.600 question to Ben and Lou.

NOTE Confidence: 0.742325126

00:55:41.600 --> 00:55:41.880 Perfect.

NOTE Confidence: 0.35969973

00:55:46.240 --> 00:55:46.360 OK,

NOTE Confidence: 0.884720502222222

00:55:53.280 --> 00:55:55.197 we we did not, we actually looked at that.

NOTE Confidence: 0.884720502222222

00:55:55.200 --> 00:55:56.418 That was one of my students

NOTE Confidence: 0.884720502222222

00:55:56.418 --> 00:55:57.760 just said I'm going to throw it

NOTE Confidence: 0.884720502222222

00:55:57.760 --> 00:55:59.320 on the on the flow cytometer.

NOTE Confidence: 0.884720502222222

00:55:59.320 --> 00:56:01.256 We don't see, we don't see changes and

NOTE Confidence: 0.884720502222222

00:56:01.256 --> 00:56:03.157 see the 58 level depending on Syn.

NOTE Confidence: 0.845768184285714

00:56:05.440 --> 00:56:09.560 Ben, thank you for a wonderful talk and be

NOTE Confidence: 0.896441123333333

00:56:09.560 --> 00:56:11.000 around for a few minutes if anyone

NOTE Confidence: 0.896441123333333

00:56:11.000 --> 00:56:12.600 has extra questions. Thank you.