WEBVTT

00:00:00.000 --> 00:00:06.384 And I want to introduce Jason Shelter.

00:00:06.390 --> 00:00:08.982 Jason is an assistant professor of

00:00:08.982 --> 00:00:11.496 surgery and received his PhD from MIT,

00:00:11.500 --> 00:00:13.876 where he worked in the laboratory

00:00:13.876 --> 00:00:16.788 of Doctor Angelika Amon in the Koch

00:00:16.788 --> 00:00:18.456 Institute for Cancer Research.

00:00:18.460 --> 00:00:20.028 After completing his PhD,

00:00:20.028 --> 00:00:21.988 he established his own research

00:00:21.988 --> 00:00:24.367 group as an independent fellow at

00:00:24.367 --> 00:00:26.292 the Cold Spring Harbor Laboratory.

00:00:26.300 --> 00:00:28.604 The Shelter Lab is broadly interested

00:00:28.604 --> 00:00:30.648 in understanding the genomic changes

00:00:30.648 --> 00:00:32.420 that drive cancer progression,

00:00:32.420 --> 00:00:33.174 particularly aneuploidy,

NOTE Confidence: 0.69964416

00:00:33.174 --> 00:00:35.436 which is found in more than

NOTE Confidence: 0.69964416

00:00:35.436 --> 00:00:37.131 90% of human tumors.

NOTE Confidence: 0.69964416

00:00:37.131 --> 00:00:37.568 Additionally,

NOTE Confidence: 0.69964416

00:00:37.568 --> 00:00:39.753 they're working to identify genomic

NOTE Confidence: 0.69964416

00:00:39.753 --> 00:00:41.722 alterations that create druggable

NOTE Confidence: 0.69964416

00:00:41.722 --> 00:00:43.678 therapeutic vulnerabilities and cancer.

NOTE Confidence: 0.69964416

00:00:43.680 --> 00:00:45.632 They have recently discovered

NOTE Confidence: 0.69964416

00:00:45.632 --> 00:00:47.584 the first ever selective

NOTE Confidence: 0.69964416

00:00:47.584 --> 00:00:49.950 inhibitor of the kinase CDK 11,

NOTE Confidence: 0.69964416

00:00:49.950 --> 00:00:53.094 and developing CDK 11 inhibition as a new

NOTE Confidence: 0.69964416

00:00:53.094 --> 00:00:56.426 strategy to treat malignancies without.

NOTE Confidence: 0.69964416

00:00:56.426 --> 00:01:00.256 Further delay Jason all yours.

NOTE Confidence: 0.959933932857143

00:01:00.730 --> 00:01:03.509 Thanks so much for the kind introduction,

NOTE Confidence: 0.959933932857143

00:01:03.510 --> 00:01:05.868 so I'm very excited to be able to share

NOTE Confidence: 0.959933932857143

00:01:05.868 --> 00:01:07.950 with you today some research my lab

NOTE Confidence: 0.959933932857143

00:01:07.950 --> 00:01:10.505 has done about off target activity of

NOTE Confidence: 0.959933932857143

00:01:10.505 --> 00:01:12.705 cancer drugs undergoing clinical trials.

NOTE Confidence: 0.959933932857143

00:01:12.710 --> 00:01:14.768 These are my disclosures and this

NOTE Confidence: 0.959933932857143

00:01:14.768 --> 00:01:16.723 project really comes from a journal

NOTE Confidence: 0.959933932857143

00:01:16.723 --> 00:01:18.811 article that I read a few years ago

NOTE Confidence: 0.959933932857143

00:01:18.877 --> 00:01:20.829 that had a statistic in it that I

NOTE Confidence: 0.959933932857143

00:01:20.829 --> 00:01:22.872 found to be just absolutely shocking.

NOTE Confidence: 0.959933932857143

00:01:22.872 --> 00:01:26.284 If you look at all drugs that enter

NOTE Confidence: 0.959933932857143

00:01:26.284 --> 00:01:28.288 clinical testing and oncology,

NOTE Confidence: 0.959933932857143

00:01:28.290 --> 00:01:30.984 97% of drug indication pairs that

NOTE Confidence: 0.959933932857143

00:01:30.984 --> 00:01:33.740 enter clinical trials fail during that

NOTE Confidence: 0.959933932857143

00:01:33.740 --> 00:01:36.386 testing and don't end up receiving

NOTE Confidence: 0.959933932857143

00:01:36.386 --> 00:01:38.606 FDA approval and this 97% failure

NOTE Confidence: 0.959933932857143

00:01:38.606 --> 00:01:40.574 rate for oncology drugs is the

NOTE Confidence: 0.959933932857143

00:01:40.574 --> 00:01:42.657 highest of any field of medicine.

NOTE Confidence: 0.959933932857143

00:01:42.660 --> 00:01:45.228 So more cancer drugs fail than

NOTE Confidence: 0.959933932857143

00:01:45.228 --> 00:01:46.940 psychiatric drugs or endocrinology,

NOTE Confidence: 0.959933932857143

00:01:46.940 --> 00:01:48.808 drugs, or infectious disease,

NOTE Confidence: 0.959933932857143

00:01:48.808 --> 00:01:50.676 drugs or anything else.

NOTE Confidence: 0.959933932857143

00:01:50.680 --> 00:01:53.864 And if you look at the proximate causes

NOTE Confidence: 0.959933932857143

00:01:53.864 --> 00:01:56.501 for trial failure, the most common,

NOTE Confidence: 0.959933932857143

00:01:56.501 --> 00:01:58.703 immediate causes that drugs run into

NOTE Confidence: 0.959933932857143

00:01:58.703 --> 00:02:01.080 are toxicity and limited efficacy.

NOTE Confidence: 0.959933932857143

00:02:01.080 --> 00:02:01.644 That is,

NOTE Confidence: 0.959933932857143

00:02:01.644 --> 00:02:03.336 the drugs have too many side

NOTE Confidence: 0.959933932857143

00:02:03.336 --> 00:02:05.338 effects for patients to safely take.

NOTE Confidence: 0.959933932857143

00:02:05.340 --> 00:02:07.276 Or maybe the patients can safely take them,

NOTE Confidence: 0.959933932857143

00:02:07.280 --> 00:02:09.513 but they have limited efficacy and they

NOTE Confidence: 0.959933932857143

00:02:09.513 --> 00:02:11.578 don't actually shrink the patient's tumor.

NOTE Confidence: 0.959933932857143

00:02:11.580 --> 00:02:13.267 And while these are kind of the.

NOTE Confidence: 0.959933932857143

00:02:13.270 --> 00:02:15.302 Proximate causes for oncology

4

NOTE Confidence: 0.959933932857143

00:02:15.302 --> 00:02:16.826 drug trial failure.

NOTE Confidence: 0.959933932857143

00:02:16.830 --> 00:02:18.615 I think the underlying reasons

NOTE Confidence: 0.959933932857143

00:02:18.615 --> 00:02:21.400 why so many drugs run into these

NOTE Confidence: 0.959933932857143

00:02:21.400 --> 00:02:23.710 problems isn't very well understood,

NOTE Confidence: 0.959933932857143

00:02:23.710 --> 00:02:25.950 and today I'm going to share some

NOTE Confidence: 0.959933932857143

00:02:25.950 --> 00:02:28.210 evidence from my lab towards one

NOTE Confidence: 0.959933932857143

00:02:28.210 --> 00:02:30.260 potential explanation for this high

NOTE Confidence: 0.959933932857143

00:02:30.260 --> 00:02:32.404 failure rate and the hypothesis that

NOTE Confidence: 0.959933932857143

00:02:32.404 --> 00:02:35.068 I'm going to argue for is that many

NOTE Confidence: 0.959933932857143

00:02:35.068 --> 00:02:37.402 drugs are entering clinical testing and

NOTE Confidence: 0.959933932857143

00:02:37.402 --> 00:02:39.543 oncology with an incorrect understanding

NOTE Confidence: 0.959933932857143

00:02:39.543 --> 00:02:41.703 of their mechanism of action,

NOTE Confidence: 0.959933932857143

00:02:41.710 --> 00:02:43.625 and I think this mischaracterization

NOTE Confidence: 0.959933932857143

00:02:43.625 --> 00:02:44.774 of cancer drugs.

NOTE Confidence: 0.959933932857143

00:02:44.780 --> 00:02:48.119 Maybe one factor by no means the only factor,

NOTE Confidence: 0.959933932857143

00:02:48.120 --> 00:02:50.440 but one factor that contributes

NOTE Confidence: 0.959933932857143

00:02:50.440 --> 00:02:52.760 to this extremely high failure

NOTE Confidence: 0.959933932857143

00:02:52.835 --> 00:02:54.927 rate for oncology therapies.

NOTE Confidence: 0.959933932857143

00:02:54.930 --> 00:02:56.246 So in my lab,

NOTE Confidence: 0.959933932857143

00:02:56.246 --> 00:02:57.891 we've been interested in using

NOTE Confidence: 0.959933932857143

00:02:57.891 --> 00:02:59.731 genetic approaches to investigate

NOTE Confidence: 0.959933932857143

00:02:59.731 --> 00:03:02.136 the mechanisms of action of

NOTE Confidence: 0.959933932857143

00:03:02.136 --> 00:03:03.990 different experimental cancer drugs,

NOTE Confidence: 0.959933932857143

00:03:03.990 --> 00:03:06.246 and by searching through the current

NOTE Confidence: 0.959933932857143

00:03:06.246 --> 00:03:08.116 literature and looking on clinicaltrials.gov,

NOTE Confidence: 0.959933932857143

00:03:08.116 --> 00:03:10.678 we put together a list of 12

NOTE Confidence: 0.959933932857143

00:03:10.678 --> 00:03:12.658 different drugs targeting 7 different

NOTE Confidence: 0.959933932857143

00:03:12.658 --> 00:03:14.633 cancer related proteins that we

NOTE Confidence: 0.959933932857143

00:03:14.633 --> 00:03:16.169 were interested in studying.

NOTE Confidence: 0.959933932857143

00:03:16.170 --> 00:03:18.516 These drugs have been used in

NOTE Confidence: 0.959933932857143

00:03:18.516 --> 00:03:20.672 about 30 different clinical trials

NOTE Confidence: 0.959933932857143

00:03:20.672 --> 00:03:23.247 targeting several 100 cancer patients.

NOTE Confidence: 0.959933932857143

00:03:23.250 --> 00:03:25.980 So six of these proteins are reported

NOTE Confidence: 0.959933932857143

00:03:25.980 --> 00:03:28.869 to be cancer genetic dependencies.

NOTE Confidence: 0.959933932857143

00:03:28.870 --> 00:03:31.005 That is the function of these proteins

NOTE Confidence: 0.959933932857143

00:03:31.005 --> 00:03:33.408 is reported to be essential for the

NOTE Confidence: 0.959933932857143

00:03:33.408 --> 00:03:35.550 growth and proliferation of cancer cells.

NOTE Confidence: 0.959933932857143

00:03:35.550 --> 00:03:36.384 For instance,

NOTE Confidence: 0.959933932857143

00:03:36.384 --> 00:03:38.469 pack four is a kinase.

NOTE Confidence: 0.959933932857143

00:03:38.470 --> 00:03:40.708 It's been reported that Pack 4

NOTE Confidence: 0.959933932857143

00:03:40.708 --> 00:03:42.595 kinase activity is essential for

NOTE Confidence: 0.959933932857143

00:03:42.595 --> 00:03:44.230 the growth of colon cancer.

NOTE Confidence: 0.959933932857143

00:03:44.230 --> 00:03:45.718 Lung cancer, breast cancer,

NOTE Confidence: 0.959933932857143

00:03:45.718 --> 00:03:47.950 and a few other cancer types.

NOTE Confidence: 0.959933932857143

00:03:47.950 --> 00:03:50.896 And because of that genetic data

NOTE Confidence: 0.959933932857143

00:03:50.896 --> 00:03:53.800 concerning pack four that motivated.

NOTE Confidence: 0.959933932857143

00:03:53.800 --> 00:03:55.924 Wiser to develop a small molecule

NOTE Confidence: 0.959933932857143

00:03:55.924 --> 00:03:56.986 pack for inhibitor

NOTE Confidence: 0.958282144444444

00:03:59.020 --> 00:04:00.995 PF 3758309 which they then

NOTE Confidence: 0.958282144444444

00:04:00.995 --> 00:04:02.575 entered into clinical testing.

NOTE Confidence: 0.958282144444444

00:04:02.580 --> 00:04:04.274 Caspase 3 is a little bit different.

NOTE Confidence: 0.958282144444444

00:04:04.280 --> 00:04:07.160 I'm going to talk about Caspase 3 separately,

NOTE Confidence: 0.958282144444444

00:04:07.160 --> 00:04:09.274 so we were interested in testing the

NOTE Confidence: 0.958282144444444

00:04:09.274 --> 00:04:11.176 mechanism of action of these drugs

NOTE Confidence: 0.958282144444444

00:04:11.176 --> 00:04:13.054 and seeing whether they killed cancer

NOTE Confidence: 0.958282144444444

00:04:13.054 --> 00:04:14.901 cells through the inhibition of these

NOTE Confidence: 0.958282144444444

00:04:14.901 --> 00:04:16.998 proteins and as a first step towards

NOTE Confidence: 0.958282144444444

00:04:16.998 --> 00:04:19.126 this process we wanted to confirm that

NOTE Confidence: 0.958282144444444

00:04:19.126 --> 00:04:21.564 the proteins these drugs were targeting

NOTE Confidence: 0.958282144444444

00:04:21.564 --> 00:04:23.639 were truly cancer genetic dependencies.

NOTE Confidence: 0.958282144444444

00:04:23.640 --> 00:04:24.770 That is, they were essential.

NOTE Confidence: 0.958282144444444

00:04:24.770 --> 00:04:27.570 For cancer growth and to investigate this,

00:04:27.570 --> 00:04:29.394 we set up a crisper competition

00:04:29.394 --> 00:04:31.400 assay to see what happened when

00:04:31.400 --> 00:04:33.215 we knocked these jeans out.

00:04:33.220 --> 00:04:34.910 To do this CRISPR assay,

00:04:34.910 --> 00:04:36.940 we transduced cancer cell lines

00:04:36.940 --> 00:04:39.812 with cast 9 and then we transduced

00:04:39.812 --> 00:04:42.486 them a second time with a guide

00:04:42.486 --> 00:04:45.060 RNA coexpressed along with GFP.

00:04:45.060 --> 00:04:47.148 This would then create a mixed

00:04:47.148 --> 00:04:48.925 population of GFP positive cells

00:04:48.925 --> 00:04:51.151 that had the guide RNA and caused

00:04:51.151 --> 00:04:53.714 mutations in the target gene and then

00:04:53.714 --> 00:04:55.579 UN transduced non fluorescent cells.

00:04:55.580 --> 00:04:57.700 We then measure the percentage

00:04:57.700 --> 00:04:59.820 of GFP cells over time.

00:04:59.820 --> 00:05:02.190 If the percent of GFP positive

NOTE Confidence: 0.958282144444444

00:05:02.190 --> 00:05:03.375 cells decreases overtime,

NOTE Confidence: 0.958282144444444

00:05:03.380 --> 00:05:05.096 that tells us that whatever gene

NOTE Confidence: 0.958282144444444

00:05:05.096 --> 00:05:07.059 the guide RNA is knocking out,

NOTE Confidence: 0.958282144444444

00:05:07.060 --> 00:05:09.328 it must be required for cancer growth

NOTE Confidence: 0.958282144444444

00:05:09.328 --> 00:05:11.900 because the GFP positive cells are dying.

NOTE Confidence: 0.958282144444444

00:05:11.900 --> 00:05:12.584 In contrast,

NOTE Confidence: 0.958282144444444

00:05:12.584 --> 00:05:14.636 if the percent of GFP positive

NOTE Confidence: 0.958282144444444

00:05:14.636 --> 00:05:16.319 cells stays about the same,

NOTE Confidence: 0.958282144444444

00:05:16.320 --> 00:05:18.070 then that's evidence that whatever

NOTE Confidence: 0.958282144444444

00:05:18.070 --> 00:05:19.820 this guide RNA is targeting,

NOTE Confidence: 0.958282144444444

00:05:19.820 --> 00:05:21.730 it isn't important for cancer

NOTE Confidence: 0.958282144444444

00:05:21.730 --> 00:05:23.640 growth because these GFP positive

NOTE Confidence: 0.958282144444444

00:05:23.703 --> 00:05:25.218 cells can grow just fine.

NOTE Confidence: 0.958282144444444

00:05:25.220 --> 00:05:27.788 So that's what the assay looked like we

NOTE Confidence: 0.958282144444444

00:05:27.788 --> 00:05:30.230 designed and cloned multiple guide RNA's.

NOTE Confidence: 0.958282144444444

00:05:30.230 --> 00:05:32.180 Targeting each of the putative

NOTE Confidence: 0.958282144444444

00:05:32.180 --> 00:05:34.130 cancer genetic dependencies we were

NOTE Confidence: 0.958282144444444

00:05:34.190 --> 00:05:36.321 interested in studying and then we

NOTE Confidence: 0.958282144444444

00:05:36.321 --> 00:05:37.983 did a bunch of competition assays

NOTE Confidence: 0.958282144444444

00:05:37.990 --> 00:05:40.209 and this is what one of these

NOTE Confidence: 0.958282144444444

00:05:40.209 --> 00:05:41.520 competition assays looks like.

NOTE Confidence: 0.958282144444444

00:05:41.520 --> 00:05:44.608 So here we're in MD AMB 231 sells

NOTE Confidence: 0.958282144444444

00:05:44.608 --> 00:05:46.153 a triple negative breast cancer

NOTE Confidence: 0.958282144444444

00:05:46.153 --> 00:05:48.179 cell line as negative controls.

NOTE Confidence: 0.958282144444444

00:05:48.180 --> 00:05:50.120 We have guide RNA's targeting

NOTE Confidence: 0.958282144444444

00:05:50.120 --> 00:05:52.628 nonessential loci. Rosa 26 and eight.

NOTE Confidence: 0.958282144444444

00:05:52.628 --> 00:05:54.788 The S1 guide RNA's targeting.

NOTE Confidence: 0.958282144444444

00:05:54.790 --> 00:05:57.526 These genes exhibit no drop out.

NOTE Confidence: 0.958282144444444

00:05:57.530 --> 00:06:00.050 As positive controls we have guide RNA's,

NOTE Confidence: 0.958282144444444

00:06:00.050 --> 00:06:02.710 targeting the essential replication genes,

NOTE Confidence: 0.958282144444444

00:06:02.710 --> 00:06:04.202 or PA3 and PC,

NOTE Confidence: 0.958282144444444

00:06:04.202 --> 00:06:06.990 and a guide RNA's targeting these genes,

NOTE Confidence: 0.958282144444444

00:06:06.990 --> 00:06:09.306 which are required for DNA replication.

NOTE Confidence: 0.958282144444444

00:06:09.310 --> 00:06:11.998 Drop out between 50 fold and 200

NOTE Confidence: 0.958282144444444

00:06:11.998 --> 00:06:14.988 fold over 5 passages in culture.

NOTE Confidence: 0.958282144444444

00:06:14.990 --> 00:06:16.970 We then looked at the effects of guide RNA's,

NOTE Confidence: 0.958282144444444

00:06:16.970 --> 00:06:18.720 targeting each of the putative

NOTE Confidence: 0.958282144444444

00:06:18.720 --> 00:06:20.120 cancer genetic dependencies that

NOTE Confidence: 0.958282144444444

00:06:20.120 --> 00:06:22.009 we were interested in studying,

NOTE Confidence: 0.958282144444444

00:06:22.010 --> 00:06:24.775 and we were really astounded when the

NOTE Confidence: 0.958282144444444

00:06:24.775 --> 00:06:27.550 guide RNA's targeting these cancer drug

NOTE Confidence: 0.958282144444444

00:06:27.550 --> 00:06:29.930 targets exhibited no dropout whatsoever.

NOTE Confidence: 0.958282144444444

00:06:29.930 --> 00:06:31.690 These guide RNAs behaved exactly

NOTE Confidence: 0.958282144444444

00:06:31.690 --> 00:06:33.450 the same as guide RNA's,

NOTE Confidence: 0.958282144444444

00:06:33.450 --> 00:06:35.382 targeting known non essential

NOTE Confidence: 0.958282144444444

00:06:35.382 --> 00:06:38.730 genes like Rosa 26 and a VS1.

NOTE Confidence: 0.958282144444444

00:06:38.730 --> 00:06:40.776 This was incredibly surprising to us

NOTE Confidence: 0.958282144444444

00:06:40.776 --> 00:06:43.145 because right now there are patients who

NOTE Confidence: 0.958282144444444

00:06:43.145 --> 00:06:45.336 are receiving anti htac 6 therapy and.

NOTE Confidence: 0.958282144444444

00:06:45.340 --> 00:06:47.338 Anti milk therapy and anti Kim.

NOTE Confidence: 0.958282144444444

00:06:47.340 --> 00:06:50.308 One therapy based on the belief that these

NOTE Confidence: 0.958282144444444

00:06:50.308 --> 00:06:52.938 proteins are required for cancer growth,

NOTE Confidence: 0.958282144444444

00:06:52.940 --> 00:06:55.784 but this experiment suggests that in

NOTE Confidence: 0.958282144444444

00:06:55.784 --> 00:06:58.126 these experimental conditions in this

NOTE Confidence: 0.958282144444444

00:06:58.126 --> 00:07:00.765 cell line we can eliminate these genes

NOTE Confidence: 0.958282144444444

00:07:00.765 --> 00:07:03.520 without any effect on cancer whatsoever.

NOTE Confidence: 0.965021968181818

00:07:03.520 --> 00:07:04.864 So this is what it looked

NOTE Confidence: 0.965021968181818

00:07:04.864 --> 00:07:06.080 like in one cell line.

NOTE Confidence: 0.965021968181818

00:07:06.080 --> 00:07:08.814 We ended up repeating this faceing 32

NOTE Confidence: 0.965021968181818

00:07:08.814 --> 00:07:10.338 different cancer cell lines from more

NOTE Confidence: 0.965021968181818

00:07:10.338 --> 00:07:12.259 than a dozen different cancer types,

NOTE Confidence: 0.965021968181818

13

00:07:12.260 --> 00:07:13.568 and in each of these experiments

NOTE Confidence: 0.965021968181818

00:07:13.568 --> 00:07:14.810 we got the same result.

NOTE Confidence: 0.965021968181818

00:07:14.810 --> 00:07:17.411 There is no drop out of the guide RNA's

NOTE Confidence: 0.965021968181818

00:07:17.411 --> 00:07:19.329 targeting these drug targets and there

NOTE Confidence: 0.965021968181818

00:07:19.329 --> 00:07:21.998 was no evidence that any of these genes

NOTE Confidence: 0.965021968181818

00:07:21.998 --> 00:07:24.434 were actually dependency in any cancer type.

NOTE Confidence: 0.965021968181818

00:07:24.440 --> 00:07:26.610 So this made us take a step back and think,

NOTE Confidence: 0.965021968181818

00:07:26.610 --> 00:07:27.780 well, is there something that

NOTE Confidence: 0.965021968181818

00:07:27.780 --> 00:07:29.480 could be going wrong in this assay?

NOTE Confidence: 0.965021968181818

00:07:29.480 --> 00:07:31.370 Could we be you know doing

NOTE Confidence: 0.965021968181818

00:07:31.370 --> 00:07:32.315 something incorrect here?

NOTE Confidence: 0.965021968181818

00:07:32.320 --> 00:07:33.624 And so we thought.

NOTE Confidence: 0.965021968181818

00:07:33.624 --> 00:07:35.609 Well, maybe with CRISPR.

NOTE Confidence: 0.965021968181818

00:07:35.609 --> 00:07:37.268 We're generating heterozygous

NOTE Confidence: 0.965021968181818

00:07:37.268 --> 00:07:40.270 mutations but not homozygous mutations.

NOTE Confidence: 0.965021968181818

00:07:40.270 --> 00:07:41.785 You know, maybe we're we're

NOTE Confidence: 0.965021968181818

00:07:41.785 --> 00:07:43.300 introducing mutations into these genes,

NOTE Confidence: 0.965021968181818

00:07:43.300 --> 00:07:45.120 but we're not really knocking

NOTE Confidence: 0.965021968181818

00:07:45.120 --> 00:07:46.576 out the total protein.

NOTE Confidence: 0.965021968181818

00:07:46.580 --> 00:07:47.976 So we thought OK,

NOTE Confidence: 0.965021968181818

00:07:47.976 --> 00:07:49.721 instead of doing this population

NOTE Confidence: 0.965021968181818

00:07:49.721 --> 00:07:50.700 based approach,

NOTE Confidence: 0.965021968181818

00:07:50.700 --> 00:07:52.722 let's make single cell Dr Knockout

NOTE Confidence: 0.965021968181818

00:07:52.722 --> 00:07:55.371 clones and be as sure as humanly

NOTE Confidence: 0.965021968181818

00:07:55.371 --> 00:07:57.426 possible that we were really

NOTE Confidence: 0.965021968181818

00:07:57.426 --> 00:08:00.160 eliminating 100% of the target protein.

NOTE Confidence: 0.965021968181818

00:08:00.160 --> 00:08:01.196 So we did that.

NOTE Confidence: 0.965021968181818

00:08:01.196 --> 00:08:03.178 We used A2 CRISPR guide RNA strategy

NOTE Confidence: 0.965021968181818

00:08:03.178 --> 00:08:05.747 where we designed to guide RNA targeting

NOTE Confidence: 0.965021968181818

00:08:05.747 --> 00:08:07.575 an upstream exon into downstream

NOTE Confidence: 0.965021968181818

00:08:07.575 --> 00:08:09.633 exon so that we could physically

NOTE Confidence: 0.965021968181818

00:08:09.633 --> 00:08:12.068 cut the gene out of the genome.

NOTE Confidence: 0.965021968181818

00:08:12.070 --> 00:08:14.009 And there would be no protein left.

NOTE Confidence: 0.965021968181818

00:08:14.010 --> 00:08:16.278 So we sorted a single cells

NOTE Confidence: 0.965021968181818

00:08:16.278 --> 00:08:17.790 that were double positive.

NOTE Confidence: 0.965021968181818

00:08:17.790 --> 00:08:20.070 That picked up both guide RNA's

NOTE Confidence: 0.965021968181818

00:08:20.070 --> 00:08:22.942 that we transduced in and then we

NOTE Confidence: 0.965021968181818

00:08:22.942 --> 00:08:24.958 verified target knockout using

NOTE Confidence: 0.965021968181818

00:08:24.958 --> 00:08:26.470 two independent antibodies.

NOTE Confidence: 0.965021968181818

00:08:26.470 --> 00:08:29.039 So for instance 1 gene we were

NOTE Confidence: 0.965021968181818

00:08:29.039 --> 00:08:31.048 interested in studying as math K14.

NOTE Confidence: 0.965021968181818

00:08:31.050 --> 00:08:32.736 This is the gene that encodes

NOTE Confidence: 0.965021968181818

00:08:32.736 --> 00:08:34.112 the kinase P38 alpha.

NOTE Confidence: 0.965021968181818

00:08:34.112 --> 00:08:36.548 We generated knockout clones and we

NOTE Confidence: 0.965021968181818

00:08:36.548 --> 00:08:38.198 verified complete target knockout

NOTE Confidence: 0.965021968181818

00:08:38.198 --> 00:08:40.520 using one antibody and then verified

NOTE Confidence: 0.965021968181818

00:08:40.520 --> 00:08:42.877 it again using a second antibody.

NOTE Confidence: 0.965021968181818

00:08:42.880 --> 00:08:44.120 So that we could be,

NOTE Confidence: 0.965021968181818

00:08:44.120 --> 00:08:44.830 you know,

NOTE Confidence: 0.965021968181818

00:08:44.830 --> 00:08:46.960 as sure as physically possible that

NOTE Confidence: 0.965021968181818

00:08:46.960 --> 00:08:49.918 we had truly eliminated all trace of

NOTE Confidence: 0.965021968181818

00:08:49.918 --> 00:08:52.790 these putative cancer drivers from the cell.

NOTE Confidence: 0.965021968181818

00:08:52.790 --> 00:08:53.120 However,

NOTE Confidence: 0.965021968181818

00:08:53.120 --> 00:08:55.100 when we tested the fitness effects

NOTE Confidence: 0.965021968181818

00:08:55.100 --> 00:08:56.530 of these knockout clones,

NOTE Confidence: 0.965021968181818

00:08:56.530 --> 00:08:58.354 we got exactly the same result

NOTE Confidence: 0.965021968181818

00:08:58.354 --> 00:09:00.643 that we got from the competition

NOTE Confidence: 0.965021968181818

00:09:00.643 --> 00:09:03.163 assays knocking out these putative

NOTE Confidence: 0.965021968181818

00:09:03.163 --> 00:09:05.079 cancer genetic dependencies had

NOTE Confidence: 0.965021968181818

00:09:05.079 --> 00:09:06.749 no effect on cancer growth.

NOTE Confidence: 0.965021968181818

00:09:06.750 --> 00:09:08.054 So here, for instance,

NOTE Confidence: 0.965021968181818

00:09:08.054 --> 00:09:09.684 is a proliferation assay in

NOTE Confidence: 0.965021968181818

17

00:09:09.684 --> 00:09:11.268 a Melanoma cell line.

NOTE Confidence: 0.965021968181818

00:09:11.270 --> 00:09:13.508 We have three map K14 knockout

NOTE Confidence: 0.965021968181818

00:09:13.508 --> 00:09:15.859 clones and then two control rows

NOTE Confidence: 0.965021968181818

00:09:15.859 --> 00:09:18.575 of 26 clones and these map K14

NOTE Confidence: 0.965021968181818

00:09:18.575 --> 00:09:20.390 knockout clones grow exactly as

NOTE Confidence: 0.965021968181818

00:09:20.390 --> 00:09:22.743 well as the rows of 26 control.

NOTE Confidence: 0.965021968181818

00:09:22.743 --> 00:09:23.076 Jones,

NOTE Confidence: 0.965021968181818

00:09:23.076 --> 00:09:25.740 we could also put these cells in soft

NOTE Confidence: 0.965021968181818

00:09:25.811 --> 00:09:28.326 Agar challenge their clonogenic ability.

NOTE Confidence: 0.965021968181818

00:09:28.330 --> 00:09:30.565 We saw no difference in

NOTE Confidence: 0.965021968181818

00:09:30.565 --> 00:09:31.906 Clonogenic ability either.

NOTE Confidence: 0.965021968181818

00:09:31.910 --> 00:09:34.898 These knockout cells grew just fine.

NOTE Confidence: 0.965021968181818

00:09:34.900 --> 00:09:35.736 So to sum up,

NOTE Confidence: 0.965021968181818

00:09:35.736 --> 00:09:37.272 a whole bunch of data that I

NOTE Confidence: 0.965021968181818

00:09:37.272 --> 00:09:38.616 don't have time to show you.

NOTE Confidence: 0.965021968181818

00:09:38.620 --> 00:09:40.804 We ended up eliminating all six

NOTE Confidence: 0.965021968181818

00:09:40.804 --> 00:09:42.260 different cancer driver genes

NOTE Confidence: 0.965021968181818

00:09:42.321 --> 00:09:44.659 that we were studying in at least

NOTE Confidence: 0.965021968181818

00:09:44.659 --> 00:09:46.379 three different cancer types each,

NOTE Confidence: 0.965021968181818

00:09:46.380 --> 00:09:48.840 and there was no fitness effect

NOTE Confidence: 0.965021968181818

00:09:48.840 --> 00:09:51.140 whatsoever that we could discuss.

NOTE Confidence: 0.965021968181818

00:09:51.140 --> 00:09:52.568 So this was a really strange

NOTE Confidence: 0.965021968181818

00:09:52.568 --> 00:09:54.472 finding to us and it made us try

NOTE Confidence: 0.965021968181818

00:09:54.472 --> 00:09:56.020 to figure out what was going on.

NOTE Confidence: 0.965021968181818

00:09:56.020 --> 00:09:57.478 So we were looking at the

NOTE Confidence: 0.965021968181818

00:09:57.478 --> 00:09:58.450 targets of 12 different

NOTE Confidence: 0.908104099333333

00:09:58.503 --> 00:10:00.375 anti cancer drugs in various stages

NOTE Confidence: 0.908104099333333

00:10:00.375 --> 00:10:01.988 of clinical development and we

NOTE Confidence: 0.908104099333333

00:10:01.988 --> 00:10:03.830 looked at these drug targets with

NOTE Confidence: 0.908104099333333

00:10:03.830 --> 00:10:05.240 multiple different CRISPR techniques.

NOTE Confidence: 0.908104099333333

00:10:05.240 --> 00:10:07.640 We did CRISPR competition assays.

NOTE Confidence: 0.908104099333333

00:10:07.640 --> 00:10:09.584 We made CRISPR knockouts,

NOTE Confidence: 0.908104099333333

00:10:09.584 --> 00:10:10.556 but concordantly.

NOTE Confidence: 0.908104099333333

00:10:10.560 --> 00:10:13.262 They both showed that we could eliminate

NOTE Confidence: 0.908104099333333

00:10:13.262 --> 00:10:15.456 these jeans without a detrimental

NOTE Confidence: 0.908104099333333

00:10:15.456 --> 00:10:17.388 effect on cancer proliferation.

NOTE Confidence: 0.908104099333333

00:10:17.390 --> 00:10:19.346 This then raised the question well,

NOTE Confidence: 0.908104099333333

00:10:19.350 --> 00:10:21.821 why were these genes believed to be

NOTE Confidence: 0.908104099333333

00:10:21.821 --> 00:10:23.850 cancer essential in the 1st place?

NOTE Confidence: 0.908104099333333

00:10:23.850 --> 00:10:25.698 And when we looked into the

NOTE Confidence: 0.908104099333333

00:10:25.698 --> 00:10:26.930 literature on these genes,

NOTE Confidence: 0.908104099333333

00:10:26.930 --> 00:10:29.346 we found the two main lines of evidence

NOTE Confidence: 0.908104099333333

00:10:29.346 --> 00:10:31.237 had identified these genes as cancer,

NOTE Confidence: 0.908104099333333

00:10:31.240 --> 00:10:32.130 essential initially.

NOTE Confidence: 0.908104099333333

00:10:32.130 --> 00:10:34.800 The first line of evidence identifying

NOTE Confidence: 0.908104099333333

00:10:34.800 --> 00:10:37.562 these genes as cancer essential were

NOTE Confidence: 0.908104099333333

00:10:37.562 --> 00:10:39.822 experiments done using RNA interference.

00:10:39.830 --> 00:10:42.092 The second line of evidence were

00:10:42.092 --> 00:10:44.589 experiments done using small molecule drugs,

00:10:44.590 --> 00:10:46.606 many of which had then gone

00:10:46.606 --> 00:10:48.590 on to enter clinical testing.

00:10:48.590 --> 00:10:50.590 So we wanted to see if we could

00:10:50.590 --> 00:10:52.253 backtrack a little and understand why

00:10:52.253 --> 00:10:54.889 we had come to such a different result

00:10:54.890 --> 00:10:57.230 than these previous experiments done.

00:10:57.230 --> 00:11:00.527 Using RNA I and small molecule drugs.

00:11:00.530 --> 00:11:02.364 So I'll first show you what we

00:11:02.364 --> 00:11:04.087 learned when we looked at some

00:11:04.087 --> 00:11:05.833 of the prior RNA I experiments.

00:11:05.840 --> 00:11:07.751 So this is an RNA I experiment

00:11:07.751 --> 00:11:09.622 published in the literature a few

00:11:09.622 --> 00:11:11.608 years ago that had identified the

00:11:11.608 --> 00:11:13.509 kinase pack for as essential for

NOTE Confidence: 0.908104099333333

00:11:13.509 --> 00:11:15.528 the growth of colon cancer cells.

NOTE Confidence: 0.908104099333333

00:11:15.528 --> 00:11:17.052 In this experiment,

NOTE Confidence: 0.908104099333333

00:11:17.052 --> 00:11:18.968 the investigators took SI

NOTE Confidence: 0.908104099333333

00:11:18.968 --> 00:11:20.520 RNA's targeting pack four.

NOTE Confidence: 0.908104099333333

00:11:20.520 --> 00:11:23.478 They introduced them into HCT 116,

NOTE Confidence: 0.908104099333333

00:11:23.480 --> 00:11:24.590 colon cancer cells,

NOTE Confidence: 0.908104099333333

00:11:24.590 --> 00:11:27.785 and they found that the SI RNAs decreased

NOTE Confidence: 0.908104099333333

00:11:27.785 --> 00:11:30.598 colon cancer cell survival data like

NOTE Confidence: 0.908104099333333

00:11:30.598 --> 00:11:33.111 this motivated Pfizer to enter a pack

NOTE Confidence: 0.908104099333333

00:11:33.111 --> 00:11:35.729 for inhibitor into clinical trials.

NOTE Confidence: 0.908104099333333

00:11:35.730 --> 00:11:37.930 We had found no fitness effect when we

NOTE Confidence: 0.908104099333333

00:11:37.930 --> 00:11:39.968 had knocked out packed 4 using CRISPR,

NOTE Confidence: 0.908104099333333

00:11:39.970 --> 00:11:42.322 so we wanted to see if we could

NOTE Confidence: 0.908104099333333

00:11:42.322 --> 00:11:43.614 recapitulate this result that

NOTE Confidence: 0.908104099333333

00:11:43.614 --> 00:11:45.546 had been published using RNA I.

00:11:45.550 --> 00:11:48.175 Two of these SI RNA constructs were

00:11:48.175 --> 00:11:49.729 commercially available and we had

00:11:49.730 --> 00:11:51.865 HCT 116 cells growing in my lab,

00:11:51.870 --> 00:11:53.970 so we purchased these siren's

00:11:53.970 --> 00:11:56.070 from this prior publication and

00:11:56.143 --> 00:11:58.207 then tested them in our cells.

00:11:58.210 --> 00:11:59.918 We transfected these siren's,

00:11:59.918 --> 00:12:02.480 the same from the prior publication

00:12:02.547 --> 00:12:03.800 into HCT 116 cells,

00:12:03.800 --> 00:12:06.110 and we could confirm by Western blot.

00:12:06.110 --> 00:12:09.160 These SI RNAs decrease protein

00:12:09.160 --> 00:12:11.845 expression as expected and we did

00:12:11.845 --> 00:12:13.840 a self survival assay and we could

00:12:13.908 --> 00:12:16.258 confirm that they killed HCT 116.

00:12:16.258 --> 00:12:17.914 Colon cancer cells exactly

00:12:17.914 --> 00:12:19.570 as had been reported.

NOTE Confidence: 0.908104099333333

00:12:19.570 --> 00:12:20.743 However, using CRISPR,

NOTE Confidence: 0.908104099333333

00:12:20.743 --> 00:12:23.480 we were also able to generate a

NOTE Confidence: 0.908104099333333

00:12:23.554 --> 00:12:25.900 pack for knockout clone in this

NOTE Confidence: 0.908104099333333

00:12:25.900 --> 00:12:27.900 exact same cancer cell line.

NOTE Confidence: 0.908104099333333

00:12:27.900 --> 00:12:30.105 So here we had a pack for knockout clone.

NOTE Confidence: 0.908104099333333

00:12:30.110 --> 00:12:32.406 You can see there's no pack for

NOTE Confidence: 0.908104099333333

00:12:32.406 --> 00:12:34.277 expression in either the control

NOTE Confidence: 0.908104099333333

00:12:34.277 --> 00:12:35.917 or the knockdown condition.

NOTE Confidence: 0.908104099333333

00:12:35.920 --> 00:12:37.999 And then when we did a self

NOTE Confidence: 0.908104099333333

00:12:37.999 --> 00:12:39.760 survival assay on these cells,

NOTE Confidence: 0.908104099333333

00:12:39.760 --> 00:12:41.920 we found that transfecting the

NOTE Confidence: 0.908104099333333

00:12:41.920 --> 00:12:44.853 pack 4 knockout cells with pack 4

NOTE Confidence: 0.908104099333333

00:12:44.853 --> 00:12:47.872 targeting SI RNA had exactly the same

NOTE Confidence: 0.908104099333333

00:12:47.872 --> 00:12:50.337 detrimental impact on colon cancer

NOTE Confidence: 0.908104099333333

00:12:50.337 --> 00:12:53.284 survival as it did in the pack for

NOTE Confidence: 0.908104099333333

00:12:53.284 --> 00:12:55.459 expressing Rosa 26 control cells.

NOTE Confidence: 0.908104099333333

00:12:55.460 --> 00:12:57.959 So these packed 4 targeting SI RNAs

NOTE Confidence: 0.908104099333333

00:12:57.959 --> 00:12:59.979 are killing colon cancer cells,

NOTE Confidence: 0.908104099333333

00:12:59.980 --> 00:13:01.816 but their ability to kill colon

NOTE Confidence: 0.908104099333333

00:13:01.816 --> 00:13:03.040 cancer cells is entirely

NOTE Confidence: 0.929160932631579

00:13:03.095 --> 00:13:04.527 independent of the expression

NOTE Confidence: 0.929160932631579

00:13:04.527 --> 00:13:06.317 of pack four because they're.

NOTE Confidence: 0.929160932631579

00:13:06.320 --> 00:13:09.134 Exactly as lethal in the control cells

NOTE Confidence: 0.929160932631579

00:13:09.134 --> 00:13:12.045 expressing pack four as they are in the pack.

NOTE Confidence: 0.929160932631579

00:13:12.050 --> 00:13:13.890 4 knockout clones that we

NOTE Confidence: 0.929160932631579

00:13:13.890 --> 00:13:14.994 generated using crisper.

NOTE Confidence: 0.929160932631579

00:13:15.000 --> 00:13:16.960 So this prior experiment was

NOTE Confidence: 0.929160932631579

00:13:16.960 --> 00:13:18.136 was totally reproducible.

NOTE Confidence: 0.929160932631579

00:13:18.140 --> 00:13:21.338 These sirens killed colon cancer cells,

NOTE Confidence: 0.929160932631579

00:13:21.340 --> 00:13:23.704 but just the interpretation was wrong

NOTE Confidence: 0.929160932631579

00:13:23.704 --> 00:13:26.319 because the toxicity of these Sir nase,

NOTE Confidence: 0.929160932631579

00:13:26.320 --> 00:13:28.120 is just entirely independent

NOTE Confidence: 0.929160932631579

00:13:28.120 --> 00:13:29.920 of pack for expression,

NOTE Confidence: 0.929160932631579

00:13:29.920 --> 00:13:32.230 and this seems to be commonly

NOTE Confidence: 0.929160932631579

00:13:32.230 --> 00:13:34.776 the case where we test SIRN as

NOTE Confidence: 0.929160932631579

00:13:34.776 --> 00:13:36.534 and SH RNA's in the literature.

NOTE Confidence: 0.929160932631579

00:13:36.540 --> 00:13:39.070 Over CRISPR derived knockout clones.

NOTE Confidence: 0.929160932631579

00:13:39.070 --> 00:13:42.310 The SI and SH RNA's may kill cancer cells,

NOTE Confidence: 0.929160932631579

00:13:42.310 --> 00:13:44.676 but it's just independent of the expression

NOTE Confidence: 0.929160932631579

00:13:44.676 --> 00:13:47.718 of the gene that they were designed against.

NOTE Confidence: 0.929160932631579

00:13:47.720 --> 00:13:49.176 The next thing that we wanted to

NOTE Confidence: 0.929160932631579

00:13:49.176 --> 00:13:50.794 figure out was what was going on

NOTE Confidence: 0.929160932631579

00:13:50.794 --> 00:13:51.974 with the small molecule drugs,

NOTE Confidence: 0.929160932631579

00:13:51.980 --> 00:13:54.563 many of which had then gone on to enter

NOTE Confidence: 0.929160932631579

00:13:54.563 --> 00:13:56.441 clinical testing and I'll show you

NOTE Confidence: 0.929160932631579

00:13:56.441 --> 00:13:58.749 what happened with one of those drugs.

NOTE Confidence: 0.929160932631579

00:13:58.750 --> 00:14:00.982 So pack one is a drug that was

NOTE Confidence: 0.929160932631579

00:14:00.982 --> 00:14:03.214 described with few years ago in a

NOTE Confidence: 0.929160932631579

00:14:03.214 --> 00:14:04.824 paper in nature chemical biology.

NOTE Confidence: 0.929160932631579

00:14:04.830 --> 00:14:07.595 It was developed as a Caspase 3

NOTE Confidence: 0.929160932631579

00:14:07.595 --> 00:14:09.677 activator compound so the apoptosis

NOTE Confidence: 0.929160932631579

00:14:09.677 --> 00:14:12.251 enzyme caspase 3 is normally present

NOTE Confidence: 0.929160932631579

00:14:12.251 --> 00:14:14.952 in an inactive procaspase state in

NOTE Confidence: 0.929160932631579

00:14:14.952 --> 00:14:17.785 the cell and pack one was developed

NOTE Confidence: 0.929160932631579

00:14:17.785 --> 00:14:20.221 to catalyze the conversion of caspase

NOTE Confidence: 0.929160932631579

00:14:20.221 --> 00:14:22.246 3 from its inactive procaspase

NOTE Confidence: 0.929160932631579

00:14:22.246 --> 00:14:25.060 state to its active caspase 3 state,

NOTE Confidence: 0.929160932631579

00:14:25.060 --> 00:14:27.076 at which point it would then

NOTE Confidence: 0.929160932631579

00:14:27.076 --> 00:14:29.116 kill cancer cells in this drug.

NOTE Confidence: 0.929160932631579

00:14:29.116 --> 00:14:30.826 Has been entered into three

NOTE Confidence: 0.929160932631579

00:14:30.826 --> 00:14:31.970 different clinical trials.

NOTE Confidence: 0.929160932631579

00:14:31.970 --> 00:14:32.368 However,

NOTE Confidence: 0.929160932631579

00:14:32.368 --> 00:14:34.756 this mechanism of action was worked

NOTE Confidence: 0.929160932631579

00:14:34.756 --> 00:14:37.221 out based on in vitro biochemistry

NOTE Confidence: 0.929160932631579

00:14:37.221 --> 00:14:40.007 and no one had described a mutation

NOTE Confidence: 0.929160932631579

00:14:40.083 --> 00:14:42.219 in Caspase 3 that conferred resistance

NOTE Confidence: 0.929160932631579

00:14:42.219 --> 00:14:44.982 to it or had assessed the effects of

NOTE Confidence: 0.929160932631579

00:14:44.982 --> 00:14:48.029 this drug in a Caspase 3 knockout cell.

NOTE Confidence: 0.929160932631579

00:14:48.030 --> 00:14:50.664 So using CRISPR we generated multiple

NOTE Confidence: 0.929160932631579

00:14:50.664 --> 00:14:53.159 Caspase 3 knockout clones and then

NOTE Confidence: 0.929160932631579

00:14:53.159 --> 00:14:55.145 we did a dose response curve.

NOTE Confidence: 0.929160932631579

00:14:55.150 --> 00:14:57.420 Examining the viability of wildtype

NOTE Confidence: 0.929160932631579

00:14:57.420 --> 00:15:00.230 and Caspase 3 knockout clones in

NOTE Confidence: 0.929160932631579

00:15:00.230 --> 00:15:02.560 different concentrations of pack one.

NOTE Confidence: 0.929160932631579

00:15:02.560 --> 00:15:04.170 So this is what it looked like

NOTE Confidence: 0.929160932631579

00:15:04.170 --> 00:15:05.260 for two control clones,

NOTE Confidence: 0.929160932631579

00:15:05.260 --> 00:15:08.880 2 clones expressing Arosa 26 guide RNA pack,

NOTE Confidence: 0.929160932631579

00:15:08.880 --> 00:15:11.680 one is a potent anti cancer agent.

NOTE Confidence: 0.929160932631579

00:15:11.680 --> 00:15:13.976 You can see it has an IC50 value

NOTE Confidence: 0.929160932631579

00:15:13.976 --> 00:15:16.128 of around one or two micromolar.

NOTE Confidence: 0.929160932631579

00:15:16.130 --> 00:15:16.467 However,

NOTE Confidence: 0.929160932631579

00:15:16.467 --> 00:15:19.500 when we did the same assay in the Caspase

NOTE Confidence: 0.929160932631579

00:15:19.577 --> 00:15:22.007 3 knockout clones that we generated,

NOTE Confidence: 0.929160932631579

00:15:22.010 --> 00:15:23.775 we ended up getting exactly

NOTE Confidence: 0.929160932631579

00:15:23.775 --> 00:15:25.187 the same drug curve.

NOTE Confidence: 0.929160932631579

00:15:25.190 --> 00:15:28.390 This drug is exactly as potent in caspase

NOTE Confidence: 0.929160932631579

00:15:28.390 --> 00:15:31.447 3 knockout clones as it is in caspase

NOTE Confidence: 0.929160932631579

00:15:31.447 --> 00:15:34.130 3 expressing Rosa 26 control clones.

NOTE Confidence: 0.929160932631579

00:15:34.130 --> 00:15:37.570 It has an IC50 value of 1 to 2 micromolar,

NOTE Confidence: 0.929160932631579

00:15:37.570 --> 00:15:39.370 regardless of whether these

NOTE Confidence: 0.929160932631579

00:15:39.370 --> 00:15:41.170 cells express caspase 3,

NOTE Confidence: 0.929160932631579

00:15:41.170 --> 00:15:42.229 so this drug,

NOTE Confidence: 0.929160932631579

00:15:42.229 --> 00:15:43.994 which entered clinical trials as

NOTE Confidence: 0.929160932631579

00:15:43.994 --> 00:15:46.149 a caspase 3 activating compound.

NOTE Confidence: 0.929160932631579

00:15:46.150 --> 00:15:48.850 Its anti cancer activity actually

NOTE Confidence: 0.929160932631579

00:15:48.850 --> 00:15:51.010 comes from something entirely

NOTE Confidence: 0.929160932631579

00:15:51.010 --> 00:15:52.974 independent of caspase 3 and this

NOTE Confidence: 0.929160932631579

00:15:52.974 --> 00:15:54.426 is actually the case for many

NOTE Confidence: 0.929160932631579

00:15:54.426 --> 00:15:56.157 of the drugs that we studied.

NOTE Confidence: 0.929160932631579

00:15:56.160 --> 00:15:58.040 So to show you a few more examples,

NOTE Confidence: 0.929160932631579

00:15:58.040 --> 00:16:00.590 HDK 6 is a histone deacetylase

NOTE Confidence: 0.929160932631579

00:16:00.590 --> 00:16:02.670 Celgene has developed each DAC.

NOTE Confidence: 0.929160932631579

00:16:02.670 --> 00:16:05.250 6 inhibitors sitter in a statin

NOTE Confidence: 0.929160932631579

00:16:05.250 --> 00:16:06.110 richelain ISTAT.

NOTE Confidence: 0.929160932631579

00:16:06.110 --> 00:16:09.150 We knocked out HDK 6 but we saw no change

NOTE Confidence: 0.820004865555556

00:16:09.228 --> 00:16:11.084 in sensitivity to these

NOTE Confidence: 0.820004865555556

00:16:11.084 --> 00:16:12.940 putative HDK 6 inhibitors.

NOTE Confidence: 0.820004865555556

00:16:12.940 --> 00:16:15.090 Milk is a cancer related

00:16:15.090 --> 00:16:16.380 kinase uncle therapy.

00:16:16.380 --> 00:16:17.940 Science is developed this drug,

00:16:17.940 --> 00:16:19.732 Novartis, developed this drug.

00:16:19.732 --> 00:16:22.420 We use CRISPR to knockout milk.

00:16:22.420 --> 00:16:25.458 We saw no change in sensitivity to

00:16:25.458 --> 00:16:27.620 these milk inhibitory compounds.

00:16:27.620 --> 00:16:29.330 So to sum up a whole bunch of data

00:16:29.330 --> 00:16:31.037 that I don't have time to show you,

00:16:31.040 --> 00:16:33.056 we found that target knockouts conferred

00:16:33.056 --> 00:16:34.813 no resistance for 12 different

00:16:34.813 --> 00:16:36.937 cancer drugs that we were studying.

00:16:36.940 --> 00:16:38.692 We made these knockouts and did

00:16:38.692 --> 00:16:40.725 these tests in at least three

00:16:40.725 --> 00:16:42.317 different cancer types each,

00:16:42.320 --> 00:16:46.020 so this kind of leaves us in an odd position.

00:16:46.020 --> 00:16:48.300 We were studying 12 different preclinical

NOTE Confidence: 0.820004865555556

00:16:48.300 --> 00:16:50.419 or clinical anti cancer drugs and

NOTE Confidence: 0.820004865555556

00:16:50.419 --> 00:16:52.571 in each of these cases we found that

NOTE Confidence: 0.820004865555556

00:16:52.630 --> 00:16:54.560 the reported mechanism of action.

NOTE Confidence: 0.820004865555556

00:16:54.560 --> 00:16:55.745 Was actually incorrect.

NOTE Confidence: 0.820004865555556

00:16:55.745 --> 00:16:58.510 This then raised the question well if

NOTE Confidence: 0.820004865555556

00:16:58.577 --> 00:17:01.111 these drugs are killing cancer cells at

NOTE Confidence: 0.820004865555556

00:17:01.111 --> 00:17:03.499 nanomolar or low micromolar potency,

NOTE Confidence: 0.820004865555556

00:17:03.500 --> 00:17:05.000 how is it they actually work?

NOTE Confidence: 0.820004865555556

00:17:05.000 --> 00:17:06.536 What is it they're actually targeting?

NOTE Confidence: 0.820004865555556

00:17:06.540 --> 00:17:08.404 We wanted to see if we could figure

NOTE Confidence: 0.820004865555556

00:17:08.404 --> 00:17:10.608 out how they were actually functioning.

NOTE Confidence: 0.820004865555556

00:17:10.610 --> 00:17:12.884 We've had the best success so

NOTE Confidence: 0.820004865555556

00:17:12.884 --> 00:17:15.598 far with one drug called O TS964.

NOTE Confidence: 0.820004865555556

00:17:15.598 --> 00:17:17.796 This is what the drug looks like.

NOTE Confidence: 0.820004865555556

00:17:17.800 --> 00:17:20.448 It was described in a paper in science

NOTE Confidence: 0.820004865555556

00:17:20.448 --> 00:17:21.864 Translational Medicine a few years

NOTE Confidence: 0.820004865555556

00:17:21.864 --> 00:17:24.149 ago as an inhibitor of a kinase called PBK,

NOTE Confidence: 0.820004865555556

00:17:24.150 --> 00:17:26.787 which is also called Pop K in the literature,

NOTE Confidence: 0.820004865555556

00:17:26.790 --> 00:17:27.765 but using CRISPR.

NOTE Confidence: 0.820004865555556

00:17:27.765 --> 00:17:29.715 We knocked out PVK and we

NOTE Confidence: 0.820004865555556

00:17:29.715 --> 00:17:31.570 saw no effect whatsoever.

NOTE Confidence: 0.820004865555556

00:17:31.570 --> 00:17:33.710 On sensitivity to this compound

NOTE Confidence: 0.820004865555556

00:17:33.710 --> 00:17:36.784 telling us that this drug O TS964

NOTE Confidence: 0.820004865555556

00:17:36.784 --> 00:17:39.586 must have some other cellular target.

NOTE Confidence: 0.820004865555556

00:17:39.590 --> 00:17:41.326 To see if we could figure out

NOTE Confidence: 0.820004865555556

00:17:41.326 --> 00:17:43.199 what this drug was actually doing,

NOTE Confidence: 0.820004865555556

00:17:43.200 --> 00:17:46.170 we used a genetic based approach

NOTE Confidence: 0.820004865555556

00:17:46.170 --> 00:17:47.430 for this approach.

NOTE Confidence: 0.820004865555556

00:17:47.430 --> 00:17:49.110 We took highly mutagenized

NOTE Confidence: 0.820004865555556

00:17:49.110 --> 00:17:50.994 colon cancer cells, HCT 116.

NOTE Confidence: 0.820004865555556

00:17:50.994 --> 00:17:53.178 They have a very high mutation rate

NOTE Confidence: 0.820004865555556

00:17:53.178 --> 00:17:54.727 because they're microsatellite unstable

NOTE Confidence: 0.820004865555556

00:17:54.727 --> 00:17:57.457 and then we expose these drugs to

NOTE Confidence: 0.820004865555556

00:17:57.526 --> 00:17:59.446 a nearly lethal concentration of

NOTE Confidence: 0.820004865555556

00:17:59.450 --> 00:18:03.046 O TS96 four such that about 99.9%

NOTE Confidence: 0.820004865555556

00:18:03.046 --> 00:18:05.538 of cells on the plate were killed.

NOTE Confidence: 0.820004865555556

00:18:05.540 --> 00:18:05.858 However,

NOTE Confidence: 0.820004865555556

00:18:05.858 --> 00:18:07.766 there were a few stragglers that

NOTE Confidence: 0.820004865555556

00:18:07.766 --> 00:18:09.487 remained when we cut these cells

NOTE Confidence: 0.820004865555556

00:18:09.487 --> 00:18:11.469 in the drug for a period of weeks

NOTE Confidence: 0.820004865555556

00:18:11.469 --> 00:18:13.387 until these cells were able to grow

NOTE Confidence: 0.820004865555556

00:18:13.387 --> 00:18:15.077 and form little micro colonies.

NOTE Confidence: 0.820004865555556

00:18:15.077 --> 00:18:17.351 We then subjected these cells to

NOTE Confidence: 0.820004865555556

00:18:17.351 --> 00:18:19.654 whole exome sequencing and when we

NOTE Confidence: 0.820004865555556

00:18:19.654 --> 00:18:22.020 did sequencing on the resistant clones,

NOTE Confidence: 0.820004865555556

00:18:22.020 --> 00:18:24.444 what we were hoping to see was a

NOTE Confidence: 0.820004865555556

00:18:24.444 --> 00:18:26.709 mutation that blocked whatever it was.

NOTE Confidence: 0.820004865555556

00:18:26.710 --> 00:18:28.600 This drug was actually targeting.

NOTE Confidence: 0.820004865555556

00:18:28.600 --> 00:18:31.090 Maybe these cells could survive a

NOTE Confidence: 0.820004865555556

00:18:31.090 --> 00:18:33.214 lethal treatment because they had

NOTE Confidence: 0.820004865555556

00:18:33.214 --> 00:18:35.189 some mutation preventing drug binding

NOTE Confidence: 0.820004865555556

00:18:35.189 --> 00:18:38.288 to whatever O TS96 or was actually doing.

NOTE Confidence: 0.820004865555556

00:18:38.290 --> 00:18:40.208 So when we did whole exome sequencing

NOTE Confidence: 0.820004865555556

00:18:40.208 --> 00:18:41.030 on these clones,

NOTE Confidence: 0.820004865555556

00:18:41.030 --> 00:18:43.333 we were really excited to see that

NOTE Confidence: 0.820004865555556

00:18:43.333 --> 00:18:45.566 every clone that we looked at had

NOTE Confidence: 0.820004865555556

00:18:45.566 --> 00:18:47.036 the same mutation in it.

NOTE Confidence: 0.820004865555556

00:18:47.040 --> 00:18:48.726 Every drug resistant clone had a

NOTE Confidence: 0.820004865555556

00:18:48.726 --> 00:18:50.779 mutation in the cyclin dependent kinase,

NOTE Confidence: 0.820004865555556

00:18:50.780 --> 00:18:51.610 CDK 11.

NOTE Confidence: 0.820004865555556

00:18:51.610 --> 00:18:54.515 They had a glycine to serine substitution,

NOTE Confidence: 0.820004865555556

00:18:54.520 --> 00:18:56.886 right smack dab in the middle of

NOTE Confidence: 0.820004865555556

00:18:56.886 --> 00:18:59.079 the CDK 11 kinase domain.

NOTE Confidence: 0.820004865555556

00:18:59.080 --> 00:19:00.740 So this immediately suggested to

NOTE Confidence: 0.820004865555556

00:19:00.740 --> 00:19:02.400 us that maybe this drug,

NOTE Confidence: 0.820004865555556

00:19:02.400 --> 00:19:05.456 which had been developed as a PDK inhibitor,

NOTE Confidence: 0.820004865555556

00:19:05.460 --> 00:19:07.208 was actually functioning through

NOTE Confidence: 0.820004865555556

00:19:07.208 --> 00:19:08.956 inhibition of CDK 11.

NOTE Confidence: 0.89901102625

00:19:08.960 --> 00:19:10.620 Instead, one potential limitation

NOTE Confidence: 0.89901102625

00:19:10.620 --> 00:19:12.552 to this is that, well,

NOTE Confidence: 0.89901102625

00:19:12.552 --> 00:19:14.456 there actually isn't a precedent for this.

NOTE Confidence: 0.89901102625

00:19:14.460 --> 00:19:17.718 CDK 11 hasn't been previously dropped,

NOTE Confidence: 0.89901102625

00:19:17.720 --> 00:19:20.312 so we wanted to see if this mutation

NOTE Confidence: 0.89901102625

00:19:20.312 --> 00:19:22.463 actually had anything to do with

NOTE Confidence: 0.89901102625

00:19:22.463 --> 00:19:25.940 sensitivity to OTS 964 in order to do that,

NOTE Confidence: 0.89901102625

00:19:25.940 --> 00:19:27.704 we wanted to see whether this mutation

NOTE Confidence: 0.89901102625

00:19:27.704 --> 00:19:29.310 that we discovered in the resistance.

NOTE Confidence: 0.89901102625

00:19:29.310 --> 00:19:31.715 Jones was actually sufficient to

NOTE Confidence: 0.89901102625

00:19:31.715 --> 00:19:33.988 confer resistance to OTS 964.

NOTE Confidence: 0.89901102625

00:19:33.988 --> 00:19:35.032 To test this,

NOTE Confidence: 0.89901102625

00:19:35.032 --> 00:19:37.120 we used a CRISPR knockin strategy

NOTE Confidence: 0.89901102625

00:19:37.189 --> 00:19:39.294 where we introduced this glycine

NOTE Confidence: 0.89901102625

00:19:39.294 --> 00:19:41.399 to serine substitution that we

NOTE Confidence: 0.89901102625

00:19:41.474 --> 00:19:43.749 recovered in drug resistant cells.

NOTE Confidence: 0.89901102625

00:19:43.750 --> 00:19:45.904 We knocked it into drug naive

NOTE Confidence: 0.89901102625

00:19:45.904 --> 00:19:48.093 cancer cells and then tested its

NOTE Confidence: 0.89901102625

00:19:48.093 --> 00:19:50.241 effects on on O TS964 sensitivity.

NOTE Confidence: 0.89901102625

00:19:50.241 --> 00:19:52.503 This is what it looked like.

NOTE Confidence: 0.89901102625

00:19:52.510 --> 00:19:54.175 Here we have four different

NOTE Confidence: 0.89901102625

00:19:54.175 --> 00:19:56.261 cancer cell lines treated with a

NOTE Confidence: 0.89901102625

00:19:56.261 --> 00:19:58.104 lethal concentration of O TS964,

NOTE Confidence: 0.89901102625

00:19:58.104 --> 00:20:00.396 with a negative control guide RNA.

NOTE Confidence: 0.89901102625

00:20:00.400 --> 00:20:02.870 Or if we just cut in the CDK 11 gene,

NOTE Confidence: 0.89901102625

00:20:02.870 --> 00:20:05.258 we have no cancer cell viability.

NOTE Confidence: 0.89901102625

00:20:05.260 --> 00:20:07.956 But if we introduce a repair template that

NOTE Confidence: 0.89901102625

00:20:07.956 --> 00:20:10.597 includes the glycine to serine substitution,

NOTE Confidence: 0.89901102625

00:20:10.600 --> 00:20:12.225 then we can restore viability

NOTE Confidence: 0.89901102625

00:20:12.225 --> 00:20:14.442 in the presence of an otherwise

NOTE Confidence: 0.89901102625

00:20:14.442 --> 00:20:16.634 lethal concentration of O TS964.

NOTE Confidence: 0.89901102625

00:20:16.634 --> 00:20:19.090 So this tells us that this mutation is

NOTE Confidence: 0.89901102625

00:20:19.156 --> 00:20:21.508 in fact both necessary and sufficient

NOTE Confidence: 0.89901102625

00:20:21.508 --> 00:20:23.730 for resistance to this compound.

NOTE Confidence: 0.89901102625

00:20:23.730 --> 00:20:25.878 We then followed this up with

NOTE Confidence: 0.89901102625

00:20:25.878 --> 00:20:26.952 some biochemical assays.

NOTE Confidence: 0.89901102625

00:20:26.960 --> 00:20:30.888 We confirmed that O TS964 inhibits CDK 11.

NOTE Confidence: 0.89901102625

00:20:30.890 --> 00:20:33.557 With an IC50 value of around 40

NOTE Confidence: 0.89901102625

00:20:33.557 --> 00:20:35.828 to 50 animal or in vitro,

NOTE Confidence: 0.89901102625

00:20:35.830 --> 00:20:38.798 and we did a cell based target engagement

NOTE Confidence: 0.89901102625

00:20:38.798 --> 00:20:40.870 assay using mass spectrometry,

NOTE Confidence: 0.89901102625

00:20:40.870 --> 00:20:43.132 we found that 100 animal or

NOTE Confidence: 0.89901102625

00:20:43.132 --> 00:20:44.724 treatment with O TS964.

NOTE Confidence: 0.89901102625

00:20:44.724 --> 00:20:47.643 It didn't bind to hundreds of other

NOTE Confidence: 0.89901102625

00:20:47.643 --> 00:20:50.099 cellular kinases, but it bound.

NOTE Confidence: 0.89901102625

00:20:50.099 --> 00:20:53.250 It caused about 70% of binding site

NOTE Confidence: 0.89901102625

00:20:53.250 --> 00:20:56.362 occlusion for CDK 11, and only CDK 11.

NOTE Confidence: 0.89901102625

00:20:56.362 --> 00:20:58.799 So from this work we concluded that

NOTE Confidence: 0.89901102625

00:20:58.799 --> 00:21:00.503 by profiling a mischaracterized

NOTE Confidence: 0.89901102625

00:21:00.503 --> 00:21:03.217 anti cancer agent we were actually

NOTE Confidence: 0.89901102625

00:21:03.217 --> 00:21:05.762 able to serendipitously discover the

NOTE Confidence: 0.89901102625

00:21:05.762 --> 00:21:10.030 first selective inhibitor of CDK 11.

NOTE Confidence: 0.89901102625

00:21:10.030 --> 00:21:12.590 So to sum up what I told you so far,

NOTE Confidence: 0.89901102625

00:21:12.590 --> 00:21:14.564 we're kind of operating in a space

NOTE Confidence: 0.89901102625

00:21:14.564 --> 00:21:16.790 in which the vast majority of new

NOTE Confidence: 0.89901102625

00:21:16.790 --> 00:21:18.770 therapies that get tested in human

NOTE Confidence: 0.89901102625

00:21:18.830 --> 00:21:21.350 patients in oncology don't end up working,

NOTE Confidence: 0.89901102625

00:21:21.350 --> 00:21:22.856 and we put together a collection

NOTE Confidence: 0.89901102625

00:21:22.856 --> 00:21:24.220 of these drugs to study.

NOTE Confidence: 0.89901102625

00:21:24.220 --> 00:21:26.299 And one thing that we found while

NOTE Confidence: 0.89901102625

00:21:26.299 --> 00:21:28.564 studying them is that many of these

NOTE Confidence: 0.89901102625

00:21:28.564 --> 00:21:30.199 drugs have actually been designed

NOTE Confidence: 0.89901102625

00:21:30.199 --> 00:21:32.434 to target proteins that have no

NOTE Confidence: 0.89901102625

00:21:32.434 --> 00:21:34.279 detectable role in cancer growth.

NOTE Confidence: 0.89901102625

00:21:34.280 --> 00:21:34.685 Furthermore,

NOTE Confidence: 0.89901102625

00:21:34.685 --> 00:21:37.520 while these drugs do kill cancer cells,

NOTE Confidence: 0.89901102625

00:21:37.520 --> 00:21:39.265 they largely kill cancer cells

NOTE Confidence: 0.89901102625

00:21:39.265 --> 00:21:41.010 through off target effects rather

NOTE Confidence: 0.89901102625

00:21:41.073 --> 00:21:43.233 than through the target that they

NOTE Confidence: 0.89901102625

00:21:43.233 --> 00:21:44.673 were initially designed against,

NOTE Confidence: 0.89901102625

00:21:44.680 --> 00:21:46.423 and I think that this can increase

NOTE Confidence: 0.89901102625

00:21:46.423 --> 00:21:48.372 the burden of side effects and the

NOTE Confidence: 0.89901102625

00:21:48.372 --> 00:21:49.802 decrease the efficacy when some

NOTE Confidence: 0.89901102625

00:21:49.802 --> 00:21:51.700 of these drugs are actually used.

NOTE Confidence: 0.89901102625

00:21:51.700 --> 00:21:53.575 We don't truly understand how

NOTE Confidence: 0.89901102625

00:21:53.575 --> 00:21:55.917 they're working or where their anti

NOTE Confidence: 0.89901102625

00:21:55.917 --> 00:21:57.517 cancer activity comes from.

NOTE Confidence: 0.89901102625

00:21:57.520 --> 00:22:00.061 Think this conclusion has a number of

NOTE Confidence: 0.89901102625

00:22:00.061 --> 00:22:02.039 important considerations and caveats though.

NOTE Confidence: 0.89901102625

00:22:02.040 --> 00:22:04.480 For instance, there could be

NOTE Confidence: 0.89901102625

00:22:04.480 --> 00:22:06.432 unrecognized cell type specificity.

NOTE Confidence: 0.89901102625

00:22:06.440 --> 00:22:07.912 We did these competitions

NOTE Confidence: 0.89901102625

00:22:07.912 --> 00:22:09.752 in 32 cancer cell lines.

NOTE Confidence: 0.89901102625

00:22:09.760 --> 00:22:11.124 We generated knockout clones

NOTE Confidence: 0.89901102625

00:22:11.124 --> 00:22:12.829 in three cancer types each,

NOTE Confidence: 0.89901102625

00:22:12.830 --> 00:22:13.328 but it was,

NOTE Confidence: 0.89901102625

00:22:13.328 --> 00:22:13.660 you know,

NOTE Confidence: 0.829353936153846

00:22:13.660 --> 00:22:16.408 physically, impossible for us to test

NOTE Confidence: 0.829353936153846

00:22:16.408 --> 00:22:19.558 every subtype of leukemia or every subtype.

NOTE Confidence: 0.829353936153846

00:22:19.560 --> 00:22:20.788 Kidney cancer in existence,

NOTE Confidence: 0.829353936153846

00:22:20.788 --> 00:22:22.630 and so we can't fully recognize

NOTE Confidence: 0.829353936153846

00:22:22.686 --> 00:22:24.376 rule out some unrecognized cell

NOTE Confidence: 0.829353936153846

00:22:24.376 --> 00:22:26.066 type specificity that hasn't been

NOTE Confidence: 0.829353936153846

00:22:26.124 --> 00:22:27.996 reported in the literature on these.

NOTE Confidence: 0.829353936153846

00:22:28.000 --> 00:22:30.267 Targets. Secondly,

NOTE Confidence: 0.829353936153846

00:22:30.267 --> 00:22:32.652 we specifically tested the hypothesis

NOTE Confidence: 0.829353936153846

00:22:32.652 --> 00:22:35.118 that these proteins are required

NOTE Confidence: 0.829353936153846

00:22:35.118 --> 00:22:37.528 for cell autonomous cancer growth,

NOTE Confidence: 0.829353936153846

00:22:37.530 --> 00:22:39.007 that is, cells going from you know,

NOTE Confidence: 0.829353936153846

00:22:39.010 --> 00:22:40.846 one cancer cell to 2:00 to 4:00 to 8:00,

NOTE Confidence: 0.829353936153846

00:22:40.850 --> 00:22:43.235 and so on, and this had been reported for

NOTE Confidence: 0.829353936153846

00:22:43.235 --> 00:22:45.468 each of the drugs that we had studied.

00:22:45.470 --> 00:22:47.126 However, if it turned out that,

00:22:47.130 --> 00:22:49.979 say, pack four had some role in

00:22:49.979 --> 00:22:52.509 angiogenesis or in immune evasion,

00:22:52.510 --> 00:22:54.890 or some other non cell autonomous process,

00:22:54.890 --> 00:22:57.260 that wouldn't be ruled out for

00:22:57.260 --> 00:22:58.840 the cell autonomous proliferation

00:22:58.905 --> 00:23:00.820 focused assays that we've done.

00:23:00.820 --> 00:23:02.686 I think a third important consideration

00:23:02.686 --> 00:23:04.679 is while our data suggests that

00:23:04.679 --> 00:23:06.399 these drugs are promiscuous and

00:23:06.399 --> 00:23:08.292 may have multiple targets in the

00:23:08.292 --> 00:23:10.161 cell just because a cancer drug is

00:23:10.170 --> 00:23:11.466 promiscuous doesn't necessarily mean

00:23:11.466 --> 00:23:13.840 that it will fail in the clinic.

00:23:13.840 --> 00:23:15.944 There are a number of drugs like sunitinib,

00:23:15.950 --> 00:23:18.920 Serafin, IB which do have multiple

NOTE Confidence: 0.829353936153846

00:23:18.920 --> 00:23:20.900 targets in the cell.

NOTE Confidence: 0.829353936153846

00:23:20.900 --> 00:23:21.610 And so,

NOTE Confidence: 0.829353936153846

00:23:21.610 --> 00:23:23.385 just because something is promiscuous

NOTE Confidence: 0.829353936153846

00:23:23.385 --> 00:23:25.418 doesn't necessarily mean that it will fail.

NOTE Confidence: 0.829353936153846

00:23:25.420 --> 00:23:25.763 However,

NOTE Confidence: 0.829353936153846

00:23:25.763 --> 00:23:28.507 I think that if our goal in cancer

NOTE Confidence: 0.829353936153846

00:23:28.507 --> 00:23:31.324 biology is to kind of reach a plateau

NOTE Confidence: 0.829353936153846

00:23:31.324 --> 00:23:33.206 of targeted precision medicine where

NOTE Confidence: 0.829353936153846

00:23:33.206 --> 00:23:35.272 you sequence a patient's tumor,

NOTE Confidence: 0.829353936153846

00:23:35.272 --> 00:23:37.502 you identify the mutations and

NOTE Confidence: 0.829353936153846

00:23:37.502 --> 00:23:39.220 amplifications and alterations and

NOTE Confidence: 0.829353936153846

00:23:39.220 --> 00:23:41.140 then design a drug cocktail based

NOTE Confidence: 0.829353936153846

00:23:41.140 --> 00:23:43.020 on that particular genetic profile

NOTE Confidence: 0.829353936153846

00:23:43.020 --> 00:23:44.658 in order to get to that level.

NOTE Confidence: 0.829353936153846

00:23:44.660 --> 00:23:47.486 I think we need to have a really good

00:23:47.486 --> 00:23:49.746 understanding of what drugs do and how

00:23:49.746 --> 00:23:52.049 their anti cancer activity actually arises.

00:23:52.050 --> 00:23:54.390 And what we'd suggest is that

00:23:54.390 --> 00:23:55.950 pre clinical genetic validation,

00:23:55.950 --> 00:23:58.488 particularly using CRISPR instead of RNA.

00:23:58.490 --> 00:24:00.682 I may help us get genetic insight into

00:24:00.682 --> 00:24:03.156 how anti cancer drugs work and may

00:24:03.156 --> 00:24:05.021 decrease the number of investigational

00:24:05.084 --> 00:24:07.009 drugs that enter clinical trials,

00:24:07.010 --> 00:24:10.328 but end up failing during clinical testing.

00:24:10.330 --> 00:24:12.290 So this is work that was done by my group.

00:24:12.290 --> 00:24:13.008 In particular,

00:24:13.008 --> 00:24:14.444 two really talented students

00:24:14.444 --> 00:24:16.470 and Lynn and Chris Giuliano.

00:24:16.470 --> 00:24:17.595 I'd like to acknowledge the

00:24:17.595 --> 00:24:18.990 funding and thank you so much,

NOTE Confidence: 0.829353936153846

00:24:18.990 --> 00:24:20.268 I'd be happy to answer any

NOTE Confidence: 0.829353936153846

00:24:20.268 --> 00:24:21.120 questions that you have.

NOTE Confidence: 0.951348497777778

00:24:24.680 --> 00:24:25.892 Thanks very much.

NOTE Confidence: 0.951348497777778

00:24:25.892 --> 00:24:28.316 I thought that was really great.

NOTE Confidence: 0.951348497777778

00:24:28.320 --> 00:24:32.100 I think you know one of the one of the

NOTE Confidence: 0.951348497777778

00:24:32.100 --> 00:24:34.871 things we're all aware of is that when

NOTE Confidence: 0.951348497777778

00:24:34.871 --> 00:24:37.163 we combine drugs that the toxicity

NOTE Confidence: 0.951348497777778

00:24:37.163 --> 00:24:40.118 goes way up and you know of course,

NOTE Confidence: 0.951348497777778

00:24:40.120 --> 00:24:42.143 much of the reason for that is

NOTE Confidence: 0.951348497777778

00:24:42.143 --> 00:24:44.208 that many of these drugs are

NOTE Confidence: 0.951348497777778

00:24:44.208 --> 00:24:46.088 promiscuous and are doing much

NOTE Confidence: 0.951348497777778

00:24:46.088 --> 00:24:48.529 more than what we need them to do.

NOTE Confidence: 0.951348497777778

00:24:48.530 --> 00:24:52.976 There's a there was a question a minute ago.

NOTE Confidence: 0.951348497777778

00:24:52.980 --> 00:24:58.460 Uh oh, so the from from Jeffrey Townsend.

NOTE Confidence: 0.951348497777778

00:24:58.460 --> 00:25:00.868 How were the original 12 drugs selected

46

NOTE Confidence: 0.951348497777778

00:25:00.868 --> 00:25:02.810 and assembled for investigation?

NOTE Confidence: 0.885461179230769

00:25:03.060 --> 00:25:06.436 Yep, so I didn't have time to discuss

NOTE Confidence: 0.885461179230769

00:25:06.436 --> 00:25:08.380 that extensively in this talk,

NOTE Confidence: 0.885461179230769

00:25:08.380 --> 00:25:10.678 but what we were interested in

NOTE Confidence: 0.885461179230769

00:25:10.680 --> 00:25:12.510 our underlying hypothesis is that

NOTE Confidence: 0.885461179230769

00:25:12.510 --> 00:25:14.747 the gold standard for knowing a

NOTE Confidence: 0.885461179230769

00:25:14.747 --> 00:25:16.805 cancer drugs mechanism of action is

NOTE Confidence: 0.885461179230769

00:25:16.805 --> 00:25:18.575 the identification of a mutation

NOTE Confidence: 0.885461179230769

00:25:18.575 --> 00:25:20.275 that confers resistance to it.

NOTE Confidence: 0.885461179230769

00:25:20.280 --> 00:25:22.290 The classic example here is Gleevec

NOTE Confidence: 0.885461179230769

00:25:22.290 --> 00:25:24.169 and the mutations in BCR ABL.

NOTE Confidence: 0.885461179230769

00:25:24.170 --> 00:25:26.234 Set block, Liebeck activity and our

NOTE Confidence: 0.885461179230769

00:25:26.234 --> 00:25:28.388 thinking was that drugs that lacked

NOTE Confidence: 0.885461179230769

00:25:28.388 --> 00:25:30.168 that level of genetic validation

NOTE Confidence: 0.885461179230769

00:25:30.168 --> 00:25:32.262 were less likely to be acting

NOTE Confidence: 0.885461179230769

00:25:32.262 --> 00:25:33.867 through an on target mechanism.

NOTE Confidence: 0.885461179230769

00:25:33.870 --> 00:25:35.400 So we selected drugs that

NOTE Confidence: 0.885461179230769

00:25:35.400 --> 00:25:37.336 specifically did not have that level

NOTE Confidence: 0.885461179230769

00:25:37.336 --> 00:25:39.066 of genetic evidence behind them.

NOTE Confidence: 0.600856868

00:25:40.760 --> 00:25:45.120 And from from Mike Hurwitz.

NOTE Confidence: 0.600856868

00:25:45.120 --> 00:25:46.260 Sort of along that line.

NOTE Confidence: 0.600856868

00:25:46.260 --> 00:25:47.814 Do you find it striking that every

NOTE Confidence: 0.600856868

00:25:47.814 --> 00:25:49.590 single one of your targets was wrong?

NOTE Confidence: 0.87856613

00:25:49.920 --> 00:25:53.140 Yeah, so for the sake of time,

NOTE Confidence: 0.87856613

00:25:53.140 --> 00:25:54.538 yeah, for the sake of time,

NOTE Confidence: 0.87856613

00:25:54.540 --> 00:25:57.172 I focused on the ones that were

NOTE Confidence: 0.87856613

00:25:57.172 --> 00:25:59.289 where we discovered that the

NOTE Confidence: 0.87856613

00:25:59.289 --> 00:26:01.554 mechanism of action was incorrect.

NOTE Confidence: 0.87856613

00:26:01.560 --> 00:26:03.968 However, we did have a few examples

NOTE Confidence: 0.87856613

00:26:03.968 --> 00:26:05.899 where we could validate it,

NOTE Confidence: 0.87856613

00:26:05.900 --> 00:26:07.700 and I'm just trying to here.

NOTE Confidence: 0.87856613

00:26:07.700 --> 00:26:09.685 I'm going to show just

NOTE Confidence: 0.87856613

00:26:09.685 --> 00:26:11.670 one example of that now.

NOTE Confidence: 0.87856613

00:26:11.670 --> 00:26:14.731 So this is not Lynn 3A.

NOTE Confidence: 0.87856613

00:26:14.731 --> 00:26:17.619 This is a drug that's been reported to

NOTE Confidence: 0.87856613

00:26:17.619 --> 00:26:19.921 function through P53 activation blocks.

NOTE Confidence: 0.87856613

00:26:19.921 --> 00:26:22.206 The interaction between MDM two

NOTE Confidence: 0.87856613

00:26:22.206 --> 00:26:25.555 and P53 we generated P53 knockout

NOTE Confidence: 0.87856613

00:26:25.555 --> 00:26:28.445 clones using crisper and when we

NOTE Confidence: 0.87856613

00:26:28.445 --> 00:26:30.370 did this drug sensitivity curve

NOTE Confidence: 0.87856613

00:26:30.370 --> 00:26:32.794 we found that a nutlin has no

NOTE Confidence: 0.87856613

00:26:32.794 --> 00:26:34.781 effect on the P53 knockout clones,

NOTE Confidence: 0.87856613

00:26:34.781 --> 00:26:37.192 while it kills the P53 expressing

NOTE Confidence: 0.87856613

00:26:37.192 --> 00:26:39.236 Rosa 26 control phones.

NOTE Confidence: 0.87856613

00:26:39.240 --> 00:26:40.878 So in general so this is.

NOTE Confidence: 0.87856613

00:26:40.880 --> 00:26:42.944 What we would expect for a drug that

NOTE Confidence: 0.87856613

00:26:42.944 --> 00:26:44.778 acts for an on target activity.

NOTE Confidence: 0.87856613

00:26:44.780 --> 00:26:46.796 You know a huge delta between the

NOTE Confidence: 0.87856613

00:26:46.796 --> 00:26:48.299 target knockouts and the target,

NOTE Confidence: 0.87856613

00:26:48.300 --> 00:26:49.710 expressing control clones,

NOTE Confidence: 0.87856613

00:26:49.710 --> 00:26:53.500 and we found a few examples of this.

NOTE Confidence: 0.87856613

00:26:53.500 --> 00:26:53.860 OK,

NOTE Confidence: 0.9160527775

00:26:54.300 --> 00:26:57.282 and I think this is the last

NOTE Confidence: 0.9160527775

00:26:57.282 --> 00:27:00.020 question from from Karen Anderson.

NOTE Confidence: 0.9160527775

00:27:00.020 --> 00:27:01.940 Did you make the searing mutant of CDK

NOTE Confidence: 0.9160527775

00:27:01.940 --> 00:27:04.469 11 and show that the inhibitor was no

NOTE Confidence: 0.9160527775

00:27:04.469 --> 00:27:06.230 longer effective in biochemical assays?

NOTE Confidence: 0.861997609

00:27:06.920 --> 00:27:08.852 So we have been doing the

NOTE Confidence: 0.861997609

00:27:08.852 --> 00:27:10.422 biochemical assays through ACR, oh,

NOTE Confidence: 0.861997609

00:27:10.422 --> 00:27:12.396 at the moment, we are not skilled

NOTE Confidence: 0.861997609

00:27:12.396 --> 00:27:14.938 in in vitro biochemistry ourselves,

NOTE Confidence: 0.861997609

00:27:14.940 --> 00:27:17.544 and so we've just done it with

NOTE Confidence: 0.861997609

00:27:17.544 --> 00:27:19.833 the the through the CR out and

NOTE Confidence: 0.861997609

00:27:19.833 --> 00:27:21.744 we'd be glad to to launch the

NOTE Confidence: 0.861997609

00:27:21.744 --> 00:27:23.280 collaboration to investigate that,

NOTE Confidence: 0.861997609

00:27:23.280 --> 00:27:24.760 because I think that would be very powerful.

NOTE Confidence: 0.880617993333333

00:27:26.060 --> 00:27:29.894 Well, I want to thank both Jason and Kurt.

NOTE Confidence: 0.880617993333333

00:27:29.900 --> 00:27:33.020 It makes me proud to have these kinds

NOTE Confidence: 0.880617993333333

00:27:33.020 --> 00:27:35.400 of presentations on my first day here.

NOTE Confidence: 0.880617993333333

00:27:35.400 --> 00:27:37.932 So thank you very, very much

NOTE Confidence: 0.880617993333333

00:27:37.932 --> 00:27:40.999 and we'll see you all next week.