And I want to introduce Jason Shelter. Jason is an assistant professor of surgery and received his PhD from MIT, where he worked in the laboratory of Doctor Angelika Amon in the Koch Institute for Cancer Research. After completing his PhD, he established his own research group as an independent fellow at the Cold Spring Harbor Laboratory. The Shelter Lab is broadly interested in understanding the genomic changes that drive cancer progression.
particularly aneuploidy,

which is found in more than

90% of human tumors.

Additionally,

they’re working to identify genomic

alterations that create druggable

therapeutic vulnerabilities and cancer.

They have recently discovered

the first ever selective

inhibitor of the kinase CDK 11,

and developing CDK 11 inhibition as a new

strategy to treat malignancies without.

Further delay Jason all yours.

Thanks so much for the kind introduction,

so I’m very excited to be able to share

some research my lab
00:01:07.950 --> 00:01:10.505 has done about off target activity of
cancer drugs undergoing clinical trials.

00:01:10.505 --> 00:01:12.705 These are my disclosures and this
project really comes from a journal
article that I read a few years ago
that had a statistic in it that I
found to be just absolutely shocking.

00:01:20.829 --> 00:01:22.872 If you look at all drugs that enter
clinical testing and oncology,
97% of drug indication pairs that
enter clinical trials fail during that
testing and don’t end up receiving
FDA approval and this 97% failure
rate for oncology drugs is the
highest of any field of medicine.
So more cancer drugs fail than psychiatric drugs or endocrinology, drugs, or infectious disease, and if you look at the proximate causes, the most common immediate causes that drugs run into are toxicity and limited efficacy. That is, the drugs have too many side effects for patients to safely take. Or maybe the patients can safely take them, but they have limited efficacy and they don’t actually shrink the patient’s tumor. And while these are kind of the proximate causes for oncology.
drug trial failure.

I think the underlying reasons why so many drugs run into these problems isn’t very well understood, and today I’m going to share some evidence from my lab towards one potential explanation for this high failure rate and the hypothesis that I’m going to argue for is that many drugs are entering clinical testing and oncology with an incorrect understanding of their mechanism of action, and I think this mischaracterization of cancer drugs. Maybe one factor by no means the only factor,
but one factor that contributes to this extremely high failure rate for oncolgy therapies. So in my lab, we’ve been interested in using genetic approaches to investigate the mechanisms of action of different experimental cancer drugs, and by searching through the current literature and looking on clinicaltrials.gov, we put together a list of 12 different drugs targeting 7 different cancer related proteins that we were interested in studying. These drugs have been used in about 30 different clinical trials.
targeting several 100 cancer patients. So six of these proteins are reported to be cancer genetic dependencies. That is the function of these proteins is reported to be essential for the growth and proliferation of cancer cells. For instance, pack four is a kinase. It's been reported that Pack 4 kinase activity is essential for the growth of colon cancer. Lung cancer, breast cancer, and a few other cancer types. And because of that genetic data concerning pack four that motivated.
Wiser to develop a small molecule pack for inhibitor PF 3758309 which they then entered into clinical testing. Caspase 3 is a little bit different. I’m going to talk about Caspase 3 separately, so we were interested in testing the mechanism of action of these drugs and seeing whether they killed cancer cells through the inhibition of these proteins and as a first step towards this process we wanted to confirm that the proteins these drugs were targeting were truly cancer genetic dependencies. That is, they were essential.
we set up a crisper competition

assay to see what happened when we knocked these jeans out.

do this CRISPR assay, we transduced cancer cell lines with cast 9 and then we transduced them a second time with a guide RNA coexpressed along with GFP. This would then create a mixed population of GFP positive cells that had the guide RNA and caused mutations in the target gene and then UN transduced non fluorescent cells. We then measure the percentage of GFP cells over time.
If the percent of GFP positive cells decreases overtime, that tells us that whatever gene the guide RNA is knocking out, it must be required for cancer growth because the GFP positive cells are dying. In contrast, if the percent of GFP positive cells stays about the same, then that’s evidence that whatever this guide RNA is targeting, it isn’t important for cancer growth because these GFP positive cells can grow just fine. So that’s what the assay looked like we designed and cloned multiple guide RNA’s.
Targeting each of the putative cancer genetic dependencies we were interested in studying and then we did a bunch of competition assays and this is what one of these competition assays looks like. So here we’re in MD AMB 231 sells a triple negative breast cancer cell line as negative controls. We have guide RNA’s targeting nonessential loci. Rosa 26 and eight. These genes exhibit no drop out. As positive controls we have guide RNA’s, targeting the essential replication genes.
or PA3 and PC, and a guide RNA’s targeting these genes, which are required for DNA replication. Drop out between 50 fold and 200 fold over 5 passages in culture. We then looked at the effects of guide RNA’s targeting each of the putative cancer genetic dependencies that we were interested in studying, and we were really astounded when the guide RNA’s targeting these cancer drug targets exhibited no dropout whatsoever. These guide RNAs behaved exactly the same as guide RNA’s, targeting known non essential genes like Rosa 26 and a VS1.
This was incredibly surprising to us because right now there are patients who are receiving anti HTAC 6 therapy and anti milk therapy and anti Kim. One therapy based on the belief that these proteins are required for cancer growth, but this experiment suggests that in these experimental conditions in this cell line we can eliminate these genes without any effect on cancer whatsoever. So this is what it looked like in one cell line. We ended up repeating this facing 32 different cancer cell lines from more than a dozen different cancer types.
and in each of these experiments we got the same result.

There is no drop out of the guide RNA’s targeting these drug targets and there was no evidence that any of these genes were actually dependency in any cancer type.

So this made us take a step back and think, could we be doing something incorrect here? We’re generating heterozygous mutations but not homozygous mutations. You know, maybe we’re...
introducing mutations into these genes, but we’re not really knocking out the total protein. So we thought OK, instead of doing this population based approach, let’s make single cell Dr Knockout clones and be as sure as humanly possible that we were really eliminating 100% of the target protein. So we did that. We used A2 CRISPR guide RNA strategy where we designed to guide RNA targeting an upstream exon into downstream exon so that we could physically
cut the gene out of the genome. And there would be no protein left. So we sorted single cells that were double positive. That picked up both guide RNA's. We transduced in and then we verified target knockout using two independent antibodies. For instance, we were interested in studying the gene that encodes the kinase P38 alpha. We generated knockout clones and we verified complete target knockout using one antibody and then verified it again using a second antibody.
So that we could be, you know, as sure as physically possible that we had truly eliminated all trace of these putative cancer drivers from the cell. However, when we tested the fitness effects of these knockout clones, we got exactly the same result that we got from the competition assays knocking out these putative cancer genetic dependencies had no effect on cancer growth. So here, for instance, is a proliferation assay in

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
different cancer driver genes that we were studying in at least three different cancer types each, and there was no fitness effect whatsoever that we could discuss. So this was a really strange finding to us and it made us try to figure out what was going on. So we were looking at the targets of 12 different anti cancer drugs in various stages of clinical development and we looked at these drug targets with multiple different CRISPR techniques.
We made CRISPR knockouts, but concordantly. They both showed that we could eliminate these jeans without a detrimental effect on cancer proliferation. This then raised the question well, why were these genes believed to be cancer essential in the first place? And when we looked into the literature on these genes, we found the two main lines of evidence had identified these genes as cancer essential initially. The first line of evidence identifying these genes as cancer essential were experiments done using RNA interference.
The second line of evidence were experiments done using small molecule drugs, many of which had then gone on to enter clinical testing. So we wanted to see if we could backtrack a little and understand why we had come to such a different result than these previous experiments done. Using RNA I and small molecule drugs, So I’ll first show you what we learned when we looked at some of the prior RNA I experiments. So this is an RNA I experiment published in the literature a few years ago that had identified the
kinase pack for as essential for the growth of colon cancer cells. In this experiment, the investigators took SI RNA’s targeting pack four. They introduced them into HCT 116, colon cancer cells, and they found that the SI RNAs decreased colon cancer cell survival data like this motivated Pfizer to enter a pack for inhibitor into clinical trials.

We had found no fitness effect when we had knocked out packed 4 using CRISPR, so we wanted to see if we could recapitulate this result that had been published using RNA I.
Two of these siRNA constructs were commercially available and we had HCT 116 cells growing in my lab, so we purchased these siren’s from this prior publication and then tested them in our cells. We transfected these siren’s, the same from the prior publication into HCT 116 cells, and we could confirm by Western blot. These siRNAs decrease protein expression as expected and we did a self survival assay and we could confirm that they killed HCT 116. Colon cancer cells exactly
as had been reported. However, using CRISPR, we were also able to generate a pack for knockout clone in this exact same cancer cell line. So here we had a pack for knockout clone. You can see there’s no pack for expression in either the control or the knockdown condition. And then when we did a self survival assay on these cells, we found that transfecting the pack 4 knockout cells with pack 4 targeting SI RNA had exactly the same detrimental impact on colon cancer survival as it did in the pack for
expressing Rosa 26 control cells.

So these packed 4 targeting SI RNAs are killing colon cancer cells,

but their ability to kill colon cancer cells is entirely independent of the expression because they’re. Exactly as lethal in the control cells expressing pack four as they are in the pack.

These sirens killed colon cancer cells, but just the interpretation was wrong.

So this prior experiment was totally reproducible.

These sirens killed colon cancer cells,
because the toxicity of these Sir nase,

is just entirely independent

and this seems to be commonly

the case where we test SIRN as

and SH RNA’s in the literature.

Over CRISPR derived knockout clones.

The SI and SH RNA’s may kill cancer cells,

but it’s just independent of the expression

of the gene that they were designed against.

The next thing that we wanted to

figure out was what was going on

with the small molecule drugs,

many of which had then gone on to enter

clinical testing and I’ll show you

what happened with one of those drugs.
So pack one is a drug that was described with few years ago in a paper in nature chemical biology. It was developed as a Caspase 3 activator compound so the apoptosis enzyme caspase 3 is normally present in an inactive pro-caspase state in the cell and pack one was developed to catalyze the conversion of caspase 3 from its inactive pro-caspase state to its active caspase 3 state, at which point it would then kill cancer cells in this drug. Has been entered into three different clinical trials.
However, this mechanism of action was worked out based on in vitro biochemistry and no one had described a mutation in Caspase 3 that conferred resistance to it or had assessed the effects of this drug in a Caspase 3 knockout cell. So using CRISPR we generated multiple Caspase 3 knockout clones and then we did a dose response curve. Examining the viability of wildtype and Caspase 3 knockout clones in different concentrations of pack one. So this is what it looked like for two control clones, 2 clones expressing Arosa 26 guide RNA pack,
one is a potent anti cancer agent.

You can see it has an IC50 value of around one or two micromolar.

However, when we did the same assay in the Caspase 3 knockout clones that we generated, we ended up getting exactly the same drug curve. This drug is exactly as potent in caspase as it is in caspase 3 expressing Rosa 26 control clones. It has an IC50 value of 1 to 2 micromolar, regardless of whether these cells express caspase 3, so this drug,
which entered clinical trials as a caspase 3 activating compound. Its anti cancer activity actually comes from something entirely independent of caspase 3 and this is actually the case for many of the drugs that we studied. So to show you a few more examples, HDK 6 is a histone deacetylase. Celgene has developed each DAC. We knocked out HDK 6 but we saw no change in sensitivity to these putative HDK 6 inhibitors. 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 these milk inhibitory compounds.

00:16:25.458 --> 00:16:27.620 So to sum up a whole bunch of data that I don’t have time to show you, we found that target knockouts conferred no resistance for 12 different cancer drugs that we were studying.

00:16:27.620 --> 00:16:29.330 so this kind of leaves us in an odd position.
We were studying 12 different preclinical or clinical anti-cancer drugs and in each of these cases we found that the reported mechanism of action was actually incorrect. This then raised the question, well if these drugs are killing cancer cells at nanomolar or low micromolar potency, how is it they actually work? What is it they're actually targeting? We wanted to see if we could figure out how they were actually functioning. We've had the best success so far with one drug called OTS964. This is what the drug looks like. It was described in a paper in Science.
A few years ago as an inhibitor of a kinase called PBK, which is also called Pop K in the literature, we knocked out PVK and we saw no effect whatsoever. On sensitivity to this compound, telling us that this drug OTS964 must have some other cellular target. To see if we could figure out what this drug was actually doing, we used a genetic based approach for this approach. We took highly mutagenized colon cancer cells, HCT 116.
They have a very high mutation rate because they’re microsatellite unstable and then we expose these drugs to a nearly lethal concentration of O TS96 four such that about 99.9% of cells on the plate were killed. However, there were a few stragglers that remained when we cut these cells in the drug for a period of weeks until these cells were able to grow and form little micro colonies. We then subjected these cells to whole exome sequencing and when we did sequencing on the resistant clones, what we were hoping to see was a...
mutation that blocked whatever it was. This drug was actually targeting.
Maybe these cells could survive a lethal treatment because they had some mutation preventing drug binding.
So when we did whole exome sequencing on these clones, we were really excited to see that every clone that we looked at had the same mutation in it. Every drug resistant clone had a mutation in the cyclin dependent kinase, CDK 11. They had a glycine to serine substitution,
right smack dab in the middle of the CDK 11 kinase domain. So this immediately suggested to us that maybe this drug, which had been developed as a PDK inhibitor, was actually functioning through inhibition of CDK 11. Instead, one potential limitation to this is that, well, there actually isn’t a precedent for this. CDK 11 hasn’t been previously dropped, so we wanted to see if this mutation actually had anything to do with sensitivity to OTS 964 in order to do that, and we wanted to see whether this mutation we discovered in the resistance.
Jones was actually sufficient to confer resistance to OTS 964. To test this, we used a CRISPR knockin strategy where we introduced this glycine to serine substitution that we recovered in drug resistant cells. We knocked it into drug naive cancer cells and then tested its effects on OTS964 sensitivity. This is what it looked like. Here we have four different cancer cell lines treated with a lethal concentration of OTS964, with a negative control guide RNA.
Or if we just cut in the CDK 11 gene, we have no cancer cell viability. But if we introduce a repair template that includes the glycine to serine substitution, then we can restore viability in the presence of an otherwise lethal concentration of O TS964. So this tells us that this mutation is in fact both necessary and sufficient for resistance to this compound. We then followed this up with some biochemical assays. We confirmed that O TS964 inhibits CDK 11. With an IC50 value of around 40 to 50 animal or in vitro, and we did a cell based target engagement.
00:20:38.798 --> 00:20:40.870 assay using mass spectrometry,
00:20:40.870 --> 00:20:43.132 we found that 100 animal or
00:20:43.132 --> 00:20:44.724 treatment with O TS964.
00:20:44.724 --> 00:20:47.643 It didn’t bind to hundreds of other
00:20:47.643 --> 00:20:50.099 cellular kinases, but it bound.
00:20:50.099 --> 00:20:53.250 It caused about 70% of binding site
00:20:53.250 --> 00:20:56.362 occlusion for CDK 11, and only CDK 11.
00:20:56.362 --> 00:20:58.799 So from this work we concluded that
00:20:58.799 --> 00:21:00.503 by profiling a mischaracterized
00:21:00.503 --> 00:21:03.217 anti cancer agent we were actually
00:21:03.217 --> 00:21:05.762 able to serendipitously discover the
00:21:05.762 --> 00:21:10.030 first selective inhibitor of CDK 11.
00:21:10.030 --> 00:21:12.590 So to sum up what I told you so far,
00:21:12.590 --> 00:21:14.564 we’re kind of operating in a space
00:21:14.564 --> 00:21:16.790 in which the vast majority of new
therapies that get tested in human patients in oncology don’t end up working,
and we put together a collection of these drugs to study.
And one thing that we found while studying them is that many of these
drugs have actually been designed to target proteins that have no
detectable role in cancer growth. Furthermore, while these drugs do kill cancer cells,
they largely kill cancer cells through off target effects rather than through the target that they
were initially designed against, and I think that this can increase
the burden of side effects and the decrease the efficacy when some of these drugs are actually used. We don’t truly understand how they’re working or where their anti-cancer activity comes from. Think this conclusion has a number of important considerations and caveats though. For instance, there could be unrecognized cell type specificity. We did these competitions in 32 cancer cell lines. We generated knockout clones in three cancer types each, but it was,
you know,

physically, impossible for us to test every subtype of leukemia or every subtype.

Kidney cancer in existence, and so we can’t fully recognize some unrecognized cell type specificity that hasn’t been reported in the literature on these.

Secondly, we specifically tested the hypothesis that these proteins are required for cell autonomous cancer growth, that is, cells going from one cancer cell to 2:00 to 4:00 to 8:00, and so on, and this had been reported for each of the drugs that we had studied.
However, if it turned out that, say, pack four had some role in angiogenesis or in immune evasion, or some other non cell autonomous process, that wouldn’t be ruled out for the cell autonomous proliferation focused assays that we’ve done. I think a third important consideration is while our data suggests that these drugs are promiscuous and may have multiple targets in the cell just because a cancer drug is promiscuous doesn’t necessarily mean it will fail in the clinic. There are a number of drugs like sunitinib,
Serafin, IB which do have multiple targets in the cell.

And so, just because something is promiscuous doesn’t necessarily mean that it will fail.

However, I think that if our goal in cancer biology is to kind of reach a plateau of targeted precision medicine where you sequence a patient’s tumor, you identify the mutations and amplifications and alterations and then design a drug cocktail based on that particular genetic profile in order to get to that level.

I think we need to have a really good
understanding of what drugs do and how
their anti cancer activity actually arises.
And what we’d suggest is that pre clinical genetic validation,
particularly using CRISPR instead of RNA. I may help us get genetic insight into how anti cancer drugs work and may decrease the number of investigational drugs that enter clinical trials, but end up failing during clinical testing. So this is work that was done by my group. In particular, two really talented students and Lynn and Chris Giuliano. 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
and assembled for investigation?

Yep, so I didn’t have time to discuss that extensively in this talk,

but what we were interested in our underlying hypothesis is that the gold standard for knowing a cancer drugs mechanism of action is the identification of a mutation that confers resistance to it.

The classic example here is Gleevec and the mutations in BCR ABL.

Set block, Liebeck activity and our thinking was that drugs that lacked that level of genetic validation were less likely to be acting.
through an on target mechanism.

So we selected drugs that specifically did not have that level of genetic evidence behind them.

And from Mike Hurwitz.

Sort of along that line.

Do you find it striking that every single one of your targets was wrong?

Yeah, so for the sake of time, we focused on the ones where we discovered that the mechanism of action was incorrect.

However, we did have a few examples where we could validate it, and I'm just trying to here.
I'm going to show just one example of that now. So this is not Lynn 3A. This is a drug that’s been reported to function through P53 activation blocks. The interaction between MDM two and P53 we generated P53 knockout clones using crisper and when we did this drug sensitivity curve we found that a nutlin has no effect on the P53 knockout clones, while it kills the P53 expressing Rosa 26 control phones. So in general so this is. What we would expect for a drug that
acts for an on target activity.

You know a huge delta between the target knockouts and the target, expressing control clones, and we found a few examples of this.

OK, and I think this is the last question from Karen Anderson.

Did you make the searing mutant of CDK 11 and show that the inhibitor was no longer effective in biochemical assays?

So we have been doing the biochemical assays through ACR, oh, at the moment, we are not skilled in vitro biochemistry ourselves, and so we’ve just done it with
00:27:17.544 --> 00:27:19.833 the the through the CR out and

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

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

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

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

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

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

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

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