We have two speakers today covering quite a diverse array of topics, which is terrific. Both internal speakers and I encourage people. If you have questions to type them into the chat. And then we’ll get those questions answered when the talks are are finished. So our first speaker is Antonio Omuro. You may you may know he is a professor of neurology and the chief of Neuro Oncology here and Clinical Leader Program leader of the shin of your family, can bring tumor center,
which is a new program here.

He received his initial medical training in Brazil,

then worked at Memorial Sloan Kettering for a while,

and began his faculty career at University of Miami.

He joined us in 2012.

He’s an international leader in their clinical care and research on brain tumors.

Leading pivotal research programs and treatment of these cancers,

the Genevier family Brain Tumor Center is a new yellow initiative for the Comprehensive, multidisciplinary brain tumor.
Karen, perhaps you might hear a little bit about that from Antonio, so Antonio, the floor is yours.

Thank you for speaking today.

Thank you very much, then super like to thank the organizers for inviting me to talk to you today and for today. Specifically, I was asked to share with you what’s happening. Our division in terms of clinical trials and how we’re tapping into Yale talent to build our portfolio, but I would also like to share with you the state of our fields and the spirit of almost like an
invitation to even more investigators and labs to join us in this task. So today we’re going to concentrate on gliomas and the reason for that is that they account for the vast majority of the brain tumors and as you can see here this is a fight chart showing all malignant intracranial tumors. The vast majority of the patients have either glioblastoma or other forms of gliomas which for the most part our IDH mutants, which account for grades two and three, others like Thomas and grades too. In three algorithms, this is 3 or 4.
by semester form as many Germans. In order rare tumors. But the bottom line here is that this even the most common tumor, which is unfortunately the great for glioma or glioblastoma, still is a relatively rare disease with only three point 1 patients for each 100,000 people. So it is again relatively rare disease. Fortunately, but it is, as you know, a very devastating disease and. The reason why this is such a charming diseases that you know the anatomic location really doesn’t help us. So these are places that presents
with these large tumors with lots of surrounding edema, an infiltrative microscopic disease. These terms are highly vascularized, so we're at the same time dealing with an uncle logic disease, but truly we're dealing with a neurologic disease as well, and you can imagine how challenging it is to manage. All of these symptoms were still trying to make a difference in terms of uncle logic treatments. Ends reflecting this challenge is the fact that the only drug that
00:03:15.634 --> 00:03:17.644 has shown to improve survival so
NOTE Confidence: 0.7932129
00:03:17.644 --> 00:03:19.601 far is this alkylating alkylating
NOTE Confidence: 0.7932129
00:03:19.601 --> 00:03:22.770 agent that is more than 20 years old.
NOTE Confidence: 0.7932129
00:03:22.770 --> 00:03:25.490 So this is the most dolomite and in
NOTE Confidence: 0.7932129
00:03:25.490 --> 00:03:27.755 controllers here is saying that the
NOTE Confidence: 0.7932129
00:03:27.755 --> 00:03:29.615 Missouri might improves both work
NOTE Confidence: 0.7932129
00:03:29.687 --> 00:03:31.907 for survival and overall survival,
NOTE Confidence: 0.7932129
00:03:31.910 --> 00:03:33.800 but even the experimental arm
NOTE Confidence: 0.7932129
00:03:33.800 --> 00:03:35.312 in the pivotal trial,
NOTE Confidence: 0.7932129
00:03:35.320 --> 00:03:38.300 which was published in 2005.
NOTE Confidence: 0.7932129
00:03:38.300 --> 00:03:41.275 Survival remained only 15 months for again,
NOTE Confidence: 0.7932129
00:03:41.280 --> 00:03:43.400 newly diagnosed disease and further
NOTE Confidence: 0.7932129
00:03:43.400 --> 00:03:46.415 analysis of this data has shown that
NOTE Confidence: 0.7932129
00:03:46.415 --> 00:03:48.875 this survivor benefit is mostly driven
NOTE Confidence: 0.7932129
00:03:48.875 --> 00:03:51.510 by tumors that have this afternoon.
NOTE Confidence: 0.7932129
00:03:51.510 --> 00:03:54.084 Check silence of the Mt gene
NOTE Confidence: 0.7932129
00:03:54.084 --> 00:03:56.323 promoter by methylation so these
NOTE Confidence: 0.7932129
00:03:56.323 --> 00:03:58.987 patients with math Laden GMT tend
NOTE Confidence: 0.7932129
00:03:58.987 --> 00:04:01.728 to respond better to Tim’s or mine,
NOTE Confidence: 0.7932129
00:04:01.730 --> 00:04:03.855 but they account for only
NOTE Confidence: 0.7932129
00:04:03.855 --> 00:04:06.024 about 30% of the patients.
NOTE Confidence: 0.7932129
00:04:06.024 --> 00:04:09.090 So for the remainder of the patients.
NOTE Confidence: 0.7932129
00:04:09.090 --> 00:04:10.865 The only real treatment that
NOTE Confidence: 0.7932129
00:04:10.865 --> 00:04:12.285 is available is radiation.
NOTE Confidence: 0.8231038
00:04:15.370 --> 00:04:18.306 And we did try a lot of agents,
NOTE Confidence: 0.8231038
00:04:18.310 --> 00:04:20.599 and here you’re looking at a slide
NOTE Confidence: 0.8231038
00:04:20.599 --> 00:04:22.691 from 2005 where we were talking
NOTE Confidence: 0.8231038
00:04:22.691 --> 00:04:25.120 about all of these clinical trials in
NOTE Confidence: 0.8231038
00:04:25.187 --> 00:04:27.477 glioblastoma and in other diseases.
NOTE Confidence: 0.8231038
00:04:27.480 --> 00:04:29.320 Testing these novel target therapist.
NOTE Confidence: 0.8231038
00:04:29.320 --> 00:04:31.078 So we’re very excited that for
NOTE Confidence: 0.8231038
the first time in would be able
to treat these patients with therapies that would carry minimal toxicities and tremendous efficacy,
but as you know, the story was much more.
All of these stars went on to fail in recurrent disease.
The sad thing is that or maybe the lucky thing for other diseases is that the majority of these drugs ended up being approved for other indications, but all of that rise in glucose
Thomas have failed. And more challenging is the fact that
we’re not really sure what is it about
the omens that all of these drugs actually fail one after the other,
is that because we are targeting the wrong targets,
maybe they’re not sufficiently relevant for Uncle Genesis,
or there are too many feedback loops and redundant pathways were now more and more learning about temporal spatial variations?
Or is it be ’cause these are the wrong drugs and?
We have problems you know,
of achieving adequate concentrations,
especially for drugs.

They are not very potent.

We do need to have better blood brain barrier penetration because a lot of these microscopic disease is behind an intact blood brain barrier.

Also, we still don’t know how to select basis for these drugs. We're still not sure if it should select based on specific mutations or should we go through transcription subgroups or not? Do any selection whatsoever and treat a large number of patients then then identify the responders and then go after the phenotypes that predict response.
So in other words, regardless of what we do, we certainly need to improve translation components within our trials, improve the science before, during, and after the trial and this is. Actually, paradigm that we have been following artificial. So the low hanging fruit is to try to use the genomic information that is now widely available. Only streamers to see if we can improve our outcomes. So as you know, global someone was the very
first tumor sequenced by the TSJ effort, and since then gene sequencing has become the norm when managing these patients and here looking at all types of biomes and these different colors here represents the different subtypes of gliomas. And you have no difficulty to see that. The genomic signatures are very distinct across the different histologies you can see here. The quintessential signature of the algal blooms, which is more penalty? Q coalition ideate mutation Sir promoter mutation and see I see and if you put P1 and here is the
quintessential signature of Astros

with guided meditation AT Rex,

Magician or lost interpretive fermentation.

And here’s this essential signatures

of global stoma.

Now we start to see Jeff Farm

tication or mutation Pete.

Then loss or mutation and lots

of formalities in CD case.

So putting those patients now,

arranging them into what kind of pathways

ended up being abnormally disturbers,

we can see the vast majority of

patients follow this cake recipe.

So basically 1000 kinase pathway with
PKU KTM Tor pathway activation and F1

you see also a lot of these patients

Abnormalities in senescence and a pop

ptosis and a lot of these patients

having a normality’s in cell cycle control.

But then when we put all of these

mutations have a track record of

being drug and what you can see is

unfortunately each of these mutations

is actually very where we’re not

being very good at identifying

drugs for those specific phenotypes

we heavily rely on basket trials.

But unfortunately basket trials
typically exclude patients with brain tumors were left with no trials or very trials that address. These questions we do have some low hanging fruits. Of course ideas mutation will talk a little bit more about that, but again, the message here is that it is very difficult to run therapy trials of these days. Because you really need to have strategies to tackle the rarity of each of these phenotypes. And adding to our challenges are how
these tumors evolve overtime and how they are heterogeneous to begin with. So this is a patient, for example, that at diagnosis she was enrolled in one of my trials of a notch inhibitor and she had a very typical signature of astrocytomas with identification interaxon to 53 mutations and several potential target targetable abnormalities with other abnormalities, but. When this patient again, she received the nearly diagnosed. Trial and then when she recovered, she was operated on again, even though she had a very small tumor. And what we found is that all of those
00:10:10.614 --> 00:10:13.307 potential target mutations are actually gone.

00:10:13.310 --> 00:10:15.150 We’re seeing some passengers here.

00:10:17.160 --> 00:10:19.761 But the reality that’s what’s driving

00:10:19.761 --> 00:10:22.155 this tumor now is actually probably about

00:10:22.160 --> 00:10:25.024 and you can imagine that if at this

00:10:25.024 --> 00:10:27.971 point in time of her disease we work

00:10:27.971 --> 00:10:30.869 Most patients do not have another brain

00:10:30.870 --> 00:10:33.145 surgery to have another sequence,

00:10:33.145 --> 00:10:35.190 so you go to archive tissue and we

00:10:35.190 --> 00:10:40.063 would have selected her for trials

00:10:40.063 --> 00:10:42.173 that probably were irrelevant for

00:10:42.173 --> 00:10:44.187 her at this point in time.

00:10:44.190 --> 00:10:44.653 Again,
those are males that we thought were present were actually gone. This is another example of potentially targetable mutations that actually were very different at the time of recurrence. Another difficult challenge are these patients here. So these are patients that we serve created. These is a result of the use of the Mozilla might that can cause mutations in mismatch repair genes at typically MSH. and what happens is that these patients with mismatch repair defects start accumulating all of these mutations and you can imagine that developing
target therapies for these folks. Is much harder. And one of the surprising findings these are actually much more common than we previously thought. So in moving forward what we’re trying to do is to again improve the science linked through the early development trials, so we more and more relying Phase Zero tries to show us if our drugs are actually getting into the brain, especially in areas with intact blood brain barrier. We also want to see if the.
The drugs are hitting their targets and we like to look at the pharmacodynamic effects in these resected specimens.

Be more and more have we have to work with their companies to have basket trials that actually include patients with our rare phenotypes.

There’s a shift towards more of a newly diagnosed disease. Be ’cause these are easier patients, and the genomics information is actually fresh, and where we’re dealing with recurrent disease, we typically like to re sample specials for target.
at least to exclude the hypermedia phenotype. And we also like to of course update the gene sequencing and the Uncle Genic trimers. Another trend in our field, this try to target these strong communications, but that’s not an easy task. And again, we’re going to talk a little bit more about that. But the vast majority of trials right now is actually trying to find alternative strategies that address more stable targets. So the low hanging fruit of stable targets is actually immuno therapies. So we do know that blue,
blasphemous do grow in a very human suppressive microenvironment.

And we have identified several emergent points that seem to be very important in this disease.

But on top of identifying the right even checkpoint, we have the challenges of the anatomic location itself. So you can imagine that it's much harder to trigger him and logical response. In the brain, which is, you know, traditionally considered the so-called sanctuary sites for the immune system.
And we have to get these email responses to act fast because these are tumors that grow very rapidly and they cause symptoms and we don’t have the luxury of waiting several months or years to react. The benefits of the email of therapies. And of course, if you’re triggering inflammatory responses in the brain, we have to deal with the risks of new log symptoms and neurotoxicity. Another important thing is. That this information potentially mimic some aggression.
so managing these patients can be challenging because we have to learn to how to recognize, see the progression versus real tumor progression on the MRI. But we did try and here you're looking at the very first results of the very first phase one trial utilizing image checkpoint inhibitors in global stoma and this was done in with VMS and in this trial we treated 40 patients both with nivolumab or two combinations of Nivola Bintulu map and what we found is that yes, the target definitely was present in the majority of patients,
so 60% of the patients had PDL one expression. But we didn’t see any brain toxicities which is good and perhaps bad because this could potentially reflect the fact that we are not achieving much and overall survival was very similar to historical controls, although some places it seemed to Mount more of an email response. But this went on to be tested in randomized trials and we are now reporting the final results of these shows and one by one they all failed to improve survival,
both newly diagnose and recurrent disease.

So we’re not giving up on immunotherapy, so I think our task now is to try to send what is that about the brain? That in spite of PDL one expression we’re not seeing any help from Anti PD one or anti PDL one therapies and I think for this question I think it is great to have a helping hand of people that study the CNS immunology and in this project what we did is to partner with Doctor David Hoffer and I’ll also Liliana Luca to look at how can we actually come up with better.
and what the heifers lab came up with is that this image of point called digit seems to be much more relevant in the brain. It was very interesting that in their studies they found a lot of teacher expression in DBMS and not so much digit expression in the quintessential inflammatory disease which is multiple sclerosis and. They went on to perform several studies utilizing, so sequencing that sort of confirmed that T cell dysfunction was being driven by digit in this particular disease. So to test this hypothesis in
patients with design,

this clinical trial where we are looking at.

A different cohorts of patients prior
to surgery where they will be treated
with either infected or anti PD one
or the combination or placebo and
then these patients will be brought to
surgery and then we will do a tumor
single cell RNA sequencing with an
axe as well as some studies to produce
some spatial validation of the findings.
And there will also follow these
patients longitudinally to see if we
can monitor what’s happening in the tumor.
By analyzing the T cells in the periphery.
So it’s a very exciting trial.
So I wish we had started the development of Inter PD one this way by understanding the science before going to more or larger studies that would end up being negative, but we’re very excited about this mechanism. Action also is important to emphasize that this combination of anti teachers and in fact PD one is very hot in the fields right now as you know it is already in phase 3IN. Non small cell lung cancer. And we’re very excited to bring this trial here to you.
00:18:17.550 --> 00:18:19.990 bit more of the immune system,
NOTE Confidence: 0.8553554
00:18:19.990 --> 00:18:23.595 we need a good models that are
NOTE Confidence: 0.8553554
00:18:23.595 --> 00:18:25.910 immunocompetent and one of the.
NOTE Confidence: 0.8553554
00:18:25.910 --> 00:18:27.815 A richness of our environment
NOTE Confidence: 0.8553554
00:18:27.815 --> 00:18:30.180 here is Doctor City chains work
NOTE Confidence: 0.8553554
00:18:30.180 --> 00:18:32.508 producing these jam models of global
NOTE Confidence: 0.8553554
00:18:32.508 --> 00:18:34.945 stomas where he can pretty much
NOTE Confidence: 0.8553554
00:18:34.945 --> 00:18:37.351 produce avatars for all of these
NOTE Confidence: 0.8553554
00:18:37.351 --> 00:18:39.740 phenotypes that I just showed you,
NOTE Confidence: 0.8553554
00:18:39.740 --> 00:18:42.008 and one of the ideas here is to see
NOTE Confidence: 0.8553554
00:18:42.008 --> 00:18:44.585 how these different phenotypes respond
NOTE Confidence: 0.8553554
00:18:44.585 --> 00:18:46.845 to these different immunotherapy's.
NOTE Confidence: 0.8553554
00:18:46.850 --> 00:18:49.268 So this is very exciting data
NOTE Confidence: 0.8553554
00:18:49.268 --> 00:18:51.744 which again illustrates how we can
NOTE Confidence: 0.8553554
00:18:51.744 --> 00:18:53.724 concomitantly to the development in
NOTE Confidence: 0.8553554
00:18:53.724 --> 00:18:56.607 the clinic to also try to understand.
Are these treatments in parallel in the lab?

Now another barrier for.

For the development of effective even responses is the work being done by the Iwasaki slab.

So Akiko has been working with Eric Song and General Thomas, and she has recently had this nature paper where they showed that.

There is a defective lymphatic drainage from the brain that you can correct utilizing the GFC.

So in her models that the combination of Veg FC and Anti PD one actually improves survival and was also
interesting that they also produce some experiments by injecting anti PD one directly into the CSF and also the results seem to be better than systemic administration of anti PD one. So this is all giving rise to another generation of characterizing and some new compounds that we hope to bring to clinic in the mid term. Now also again another important barrier in Spanish solid tumors, but particularly in glomus, is the role of tumor associated macrophages and how they produce these emails. and one of the ways that we could
00:20:26.548 --> 00:20:28.883 potentially intervene in this was

00:20:28.883 --> 00:20:31.668 discovered by an item here at go,

00:20:31.670 --> 00:20:34.729 where she’s looking at the role of

00:20:34.729 --> 00:20:36.975 this little Robo one, signaling

00:20:36.975 --> 00:20:40.005 which seems to attract and polarize.

00:20:40.010 --> 00:20:42.320 Save Microfridge is in in the

00:20:42.320 --> 00:20:43.475 brain microenvironment and

00:20:43.475 --> 00:20:44.709 Livingstone my confirming.

00:20:44.710 --> 00:20:47.846 And when she did experiments to knock down,

00:20:47.850 --> 00:20:50.587 slid to, or to block this pathway,

00:20:50.590 --> 00:20:52.590 she achieved better immune responses

00:20:52.590 --> 00:20:54.910 and inflammation of anti PD one.

00:20:54.910 --> 00:20:57.202 She had a really significant improvement

00:20:57.202 --> 00:21:00.389 in survival or in this tumor bearing mice.

00:21:00.390 --> 00:21:03.270 So the idea here is now to generate

NOTE Confidence: 0.7626056

34
enter Robo Nanobodies one of the barriers project would be then how can we get this number? At least to penetrate into the brain. And since she’s very resourceful, she has the answer. It looks like. If you block antibodies, if you use antibodies blocking this receptor called UNC 5B, you conserve produce an on demand blood brain barrier opening, so this is less a few hours and it’s great for drugs up to 40 kilodaltons. So the idea here is that if this is successful, we could combine this.
These agents with many of the chemotherapies in order target therapies that we are trying to use to treat these patients in a more efficient way. And overcome the problem of living there countries so very exciting work that we hope to see more of. No moving on into. It’s still sticking to the Mockingbird, but moving on to partnerships with pharma. One of the our partnerships is with this drug. This company called In Pharmaceuticals and these folks have discovered a novel receptor with within the.
Alpha V beta three integrin that is started by this FP PMT drug that seems to have an amazing activity in their mouse models. Really melting the mice. And this was the first. Now we're now designing the 1st in human trial here GAIL, that will start in a couple of months. But to understand this better we did bring Yellow Labs into the mix to better define how is this rug really working and who are the best candidates by understand a little bit more about the effects on cell invasion signaling networks.
and gene expression.

So one of the assets that.

We're realizing in partnership with

under left ankle is looking at these.

The use of his integrated platform,

The so called race essay

which is a disrupted analysis of cell

phenotype extremes where he uses

the cell migration as a surrogate

the cell migration as a surrogate

marker of tumor aggressiveness and

and then you can test the multiple

and then you can test the multiple

drugs utilizing this essay as

drugs utilizing this essay as a form of drug screening,

and he's applying this rug.

Your days were very interesting results
and we hope to then identify partners.

Which are the best genomic candidates and then see if we can optimize the trial as we go by in reaching with either best candidates or potentially novel combinations.

So again, that’s just to illustrate that it’s very important to really involve our laboratories.

Even in trials that are being conducted by pharma.

Now sticking again, not now moving on to other more stable targets, and one of them is ideas with patient
and this story came out of Doctor Kendra’s lab where he found it ideas.

Mutations change DNA repair through the production of two hydroxy obliterate, which is the byproduct of this mutation, and this results in sort of brokenness that then can be targeted by PARP inhibitors.

So he has several clinical trials of these park inhibitors.

So he has several clinical trials of these park inhibitors.

And we are now hoping to see if this will actually improve outcomes for these patients.

Also, again sticking to the DNA repair thing, we recently submitted a United team led by Mayo Clinic and John Jennifer Correa.
in partnership with even colonies.

Doctor Bindra and I.

So we have two projects.

One is trying to optimize MDM two inhibition for these patients and 80 Rd in ambition for these patients.

And this will again bring two other Phase 0 slash 1 clinical trials.

Now we don’t have time to review all of our portfolio, but we do have partnerships with industry for opening other tries to fill in gaps in our portfolio.

Doctor Blundin has activated the Agile trial which is a multi drug
mult arm clinical trial that is happening worldwide so we have access to these drugs for our patients and have a bunch of other choice. But the theme here is really to focus on early therapeutic development and then participating inside cooperative groups. Please, for those extremely rare phenotypes, for example, byref mutant craniopharyngioma switch, again very difficult to find patience and for those we do need to partner with other places around the country. And I could go on and on talking about all of the years signs
that is going in brain tumors.
I select a few stories that are closest to clinic,
but all these people in this picture and many others that I’m not even mentioning today are producing amazing size that we can actually use into our portfolio and bring it in anymore. Let’s say intelligent trials ranging from data science, junior imaging and all sorts of therapeutic approaches.
So in conclusion, so we’re lucky enough to have this unique breath of scientific expertise. Our focus is really on investigating
shaded trials that are home grown
and our other focuses on early stage
development with former partners,
but also bringing along our
own labs email collagen,
a repair have emerged as leading teams,
but here there we have many patents about,
although many are not ready
for complication and need a
lot of help for development.
We certainly need more work
on existing available drugs,
for example coming from Seatac
and Pharma and a lot of work in
functional genomics so that we
can figure out finally how to target these undruggable targets. So that I would like to finish by thanking all of the people. So when we talk clinical trials, really the merit is all of others of the labs of the all of the infrastructure. I would also like to acknowledge our division attendings and aips, or actually managing treating these patients in the trials. I would like to thank again the Cito staff. They’re going through rough times, but right Decker is navigating and it’s going to get us out of this situation. A big thanks to the PRC reviewers.
'cause one of my hats is actually as the Pearcey chair and we acknowledge along with Barbara Burtness that there was a lot of work that goes into this and that I would like to thank them publicly at this opportunity. Lots of things. So why CCI that help us with investigating share clinical trials. All of the people that have been enabling this research and finally a big thank to the YCC and Smile leadership with more Pickens.
of our clinical trials portfolio.

Last but not least, again,

I would like to thank the show her family for their generous gift.

In fact, then I’m not going to talk about this today because we’re still working on the details,

but the word is out of the receive a generous gift from that foundation,

and we’re hoping to put together a nice program that will again enable and expand on our research efforts.

Thank you very much and I’ll take some points if you have time.

OK, thank you very much Antonio.

Very interesting work.
Are there any questions that people want to enter into the chat?

While we’re waiting, I have a quick question. You mentioned this idea of opening up the bread. The blood brain barrier by targeting a molecule. Is it worth going back to some of the earlier drugs that weren’t terribly effective to see that whether or not that might help them work better? Yes, I think there is a whole list of drugs that perhaps will need to be revisited.
Although most of these drugs would actually be again in rare phenotypes, because I think those are, we still need to select them by those specific mutations. The problem of copy number remains regardless of flipping connectors. I don’t think blood brain barrier penetration was the reason why we couldn’t target EGFR amplification or Pete and loss. I think that is a different question, but if we are to even answer those we still need. This kind of approach, ’cause it makes our life so much easier.

Are there any questions from the audience?
I was also struck by the lots of different mutations upon recurrence. He showed. What is that thought to be due to is just so high perforation rate of these tumors. Yeah, well, I think so. First of all, these tumors are very heterogeneous to begin with, right? So these are guns that are were there to begin with, but it looks like the treatment process ends up eliminating a lot of this so called cancer associated mutations. Another unknown mutations emerge and also a lot of these are actually epigenetic.
So there’s a whole line of research trying to then understand this and more canals are interested in in that line of research and other labs to see how we can target these tumors at recurrence that are sort of, very simple from a genomic standpoint, but not so simple at the epigenetic level. Well, thank you very much. Very interesting.
Harris professor of Chemistry received his PhD at Harvard and then post Doc at University of Illinois, and he’s interested in natural product’s particular products that affect the synthesis or damaged DNA. And he’s received numerous multiple Young Investigator awards and working with Jason Crawford is a terrific collaboration. Looking at them, the metabolites made by the human microbiota and identified some of them that actually damaged DNA and therefore contribute to cancer.
So Seth, we're looking forward to hearing about your work.

Thank you.

OK, thanks Dan, thanks for the introduction.

And thanks to all to everyone for the invocation.

I will talk today about work we've been doing in the human microbiome, but actually ignore it.

Just calling on ever. Snap because.

Cave against drug resistant TMZ resistant.

GBM that we're very excited about but that will be a story for another day.

And so right. So today I'll talk
NOTE Confidence: 0.88522273
00:32:51.572 --> 00:32:53.928 about a project that’s been ongoing
NOTE Confidence: 0.88522273
00:32:53.928 --> 00:32:56.469 in my group for about 6 years.
NOTE Confidence: 0.88522273
00:32:56.470 --> 00:32:59.668 And we’ve been looking to understand
NOTE Confidence: 0.88522273
00:32:59.668 --> 00:33:03.200 the molecular basis of a carcinogen
NOTE Confidence: 0.88522273
00:33:03.200 --> 00:33:05.820 carcinogenic phenotype that was
NOTE Confidence: 0.88522273
00:33:05.820 --> 00:33:09.048 observed from certain gut bacteria so.
NOTE Confidence: 0.88522273
00:33:09.048 --> 00:33:11.631 I’ll go through sort of the sequence
NOTE Confidence: 0.88522273
00:33:11.631 --> 00:33:14.631 of events to kind of outline sequence
NOTE Confidence: 0.88522273
00:33:14.631 --> 00:33:17.260 of discoveries to outline the problem,
NOTE Confidence: 0.88522273
00:33:17.260 --> 00:33:20.923 and so in 2006 this was the paper that
NOTE Confidence: 0.88522273
00:33:20.923 --> 00:33:24.825 set off a lot of interest in this area.
NOTE Confidence: 0.88522273
00:33:24.830 --> 00:33:27.920 Eric Oswald and coworkers identified
NOTE Confidence: 0.88522273
00:33:27.920 --> 00:33:31.010 certain strains of commensal and
NOTE Confidence: 0.88522273
00:33:31.102 --> 00:33:34.861 pathogenic E coli that had a biosynthetic
NOTE Confidence: 0.88522273
00:33:34.861 --> 00:33:38.268 gene cluster known as the CLB cluster.
NOTE Confidence: 0.88522273
So by that I mean that gene genetic locus contains the coding for enzymes that make a secondary metabolite and he took these CLB containing bacteria and did a transient infection. HeLa cells with them and then looked at the effect on the cells, and he found that they underwent cell cycle arrest. Meglos cytosis and using a comet assay. Another Gamage to XD he saw that they accumulated double strand breaks in their DNA. And so this is a very interesting phenotype. It’s not the first time. Microbes, have, you know,
produced Gina toxins,
but it was a very interesting example and I’ll come to in a second wait.
Why it’s attracting so much attention?
Subsequent to that report,
there’s been numerous studies trying to ascertain whether or not there is a role for these bacteria in colorectal cancer formation and from the same group in 2010.
It was shown that in
intestinal loop models of.
My step or infected with CLB bacteria they observe DNA damage in vivo.
They observed gamma H2 X they observed
increased mutations in the HP RT&TK loci and then also hyperproliferation following exposure to the bacteria.

So they seem to be driving tumorigenesis and there were subsequent studies following up looking at similar types of in vivo effects.

So using IL.

Knockout mice,

it was shown that infection with these bacteria leads to a higher rate of tumor formation and then there were three groups that did.

Meta analysis of samples from CRC patients and what we find is that about 60 to 70% of
CRC patients have these bacteria and that's versus about 20% in the healthy population. And the other sort of bit is that the preponderance of these bacteria tracks with the severity of the cancer, so people with more advanced CRC were at the high end of that correlation, whereas people with early stage CRC were more at the lower end. And so it wasn’t really until last year that a causal relationship was unequivocally established. There were two studies from mayor Boxtel and Cleavers and then Boxtel and Cleavers an
in the Cleavers study.

They generated an organoid and infected that organoid chronically for about three or four months with the CLB positive bacteria and what they showed is that you get the mutational signature transformation and proliferation. We also find that that mutational signature is found. Enriched in in CRC patients as well, and so the mayor study came to similar conclusions, and essentially these two papers you know, this is a rare example in the microbiome where you actually establish causation. So these two papers brought this
phenotype to the two sort of a causal level and what my lab has been trying to do of course, is understand the molecular basis for all of this OK and so. Oswald, in his initial paper, had done a series of very nice and robust control experiments to establish that this genotoxic phenotype. Is due to the final biosynthetic product of the CLB cluster. In other words, if one modifies any of the enzymes in the CLB pathway, you lose this genotoxic phenotype OK, and so the implication then is that
it’s the fully elaborated molecule.
That is the active toxin, not something in route to another another product.
And we call that molecule Coley bactine.
And so the field basically set out to do what we do best, which is isolate compounds and the classic way of isolating natural product secondary metabolites is to culture the Organism of interest. In the case of bacterial secondary metabolite, you might grow it in liquid culture, growing on scale. Extract start to fractionate by
HPLC and then we typically do.

It’s known as activity guided fractionation, where you’re essentially testing each of these fractions for a particular phenotype. And then you keep purifying and testing until you get to a single compound and you characterize it.

The problem is that this approach does not work for Kohli. Bakhtin, OK, so the molecule is very unstable. It is very difficult to get the bacteria to express the CLB pathway ex vivo.

And what we find is that because of the.
primarily anaerobic environment of the gut.

The molecule actually undergoes oxidative degradation when you attempt to isolate it.

Sort of on the bench under air, and just to.

Give you an example of how challenging this is.

This is not work from our own laboratory.

They’ve been pursuing Cali, backed in in the isolated this.

Molecule here in 2019 they obtained 50 micrograms from a 2000 liter fermentation.

If anyone can imagine that, so we’re talking about literally vanishingly small quantities.
And they advanced this molecule as a candidate calling back, and unfortunately this was derived from a triple mutant Frankenstein like bacteria, and I wrote a commentary. If you’re interested on this at the general thinking in the field is this probably not relevant. But the point is, these are the links that people are willing to go to try and isolate these molecules. And so, how do we approach this? So, as Dan mentioned,
00:40:48.452 --> 00:40:51.080 we’ve been collaborating with Jason Crawford.
NOTE Confidence: 0.8315405
00:40:51.080 --> 00:40:53.702 Jason is one of the leaders
NOTE Confidence: 0.8315405
00:40:53.702 --> 00:40:55.013 in understanding Kohli,
NOTE Confidence: 0.8315405
00:40:55.020 --> 00:40:56.184 backed in biosynthesis.
NOTE Confidence: 0.8315405
00:40:56.184 --> 00:40:59.443 And So what we’ve been doing is really
NOTE Confidence: 0.8315405
00:40:59.443 --> 00:41:01.803 taking knowledge from the biosynthetic
NOTE Confidence: 0.8315405
00:41:01.803 --> 00:41:05.086 pathway and trying to infer what types
NOTE Confidence: 0.8315405
00:41:05.086 --> 00:41:07.720 of substructures might be within Kohli.
NOTE Confidence: 0.8315405
00:41:07.720 --> 00:41:10.160 Backed in itself and how
NOTE Confidence: 0.8315405
00:41:10.160 --> 00:41:11.624 those might interact.
NOTE Confidence: 0.8315405
00:41:11.630 --> 00:41:14.526 With DNA and so one of the sort
NOTE Confidence: 0.8315405
00:41:14.526 --> 00:41:17.778 of models that came out of these
NOTE Confidence: 0.8315405
00:41:17.778 --> 00:41:20.208 biosynthetic studies is that you
NOTE Confidence: 0.8315405
00:41:20.305 --> 00:41:23.135 have these fully linear products
NOTE Confidence: 0.8315405
00:41:23.135 --> 00:41:26.470 offloaded from the PKS assembly line.
NOTE Confidence: 0.8315405
00:41:26.470 --> 00:41:28.970 There’s a searing protease that
00:41:28.970 --> 00:41:32.179 removes this residue and blew this ACL.
00:41:32.180 --> 00:41:34.230 Asparagine residue.
00:41:34.230 --> 00:41:36.250 That generates a primary amine,
00:41:36.250 --> 00:41:38.742 and once you format that can start
00:41:38.742 --> 00:41:41.666 to wrap up and ultimately lead to
00:41:41.666 --> 00:41:44.324 this compound on the bottom here,
00:41:44.330 --> 00:41:46.784 which has a cyclopropane ring in
00:41:46.784 --> 00:41:49.578 conjugation with with it with the Alpha,
00:41:49.580 --> 00:41:51.575 beta unsaturated иммиг and for
00:41:51.575 --> 00:41:54.042 those in the audience that have
00:41:54.042 --> 00:41:55.638 worked with Gina toxins,
00:41:55.640 --> 00:41:57.660 you know that these electrophilic
00:41:57.660 --> 00:41:59.276 cyclopropane’s are not uncommon.
00:41:59.280 --> 00:42:02.264 This is a sort of a pharmacophore that’s
00:42:02.264 --> 00:42:05.460 found in a variety of genotoxic natural.
Products and so this was, you know, sort of very logically following from that type of precedent. The problem is that no one had isolated these imines. No one had any spectroscopic data on them. All we had was this. This kind of mechanistic hypothesis. And so we set out to make it, and I'm not going to have time to go through all of the synthetic work that went into developing these roots. But the key steps are shown here. So we start from this linear precursor and what we find is that if we...
00:42:40.519 --> 00:42:42.500 concentrate this down from dilute acid,
NOTE Confidence: 0.8315405
00:42:42.500 --> 00:42:45.148 we can get this.
NOTE Confidence: 0.8315405
00:42:45.150 --> 00:42:46.860 Carbon and nitrogen to condense
NOTE Confidence: 0.8315405
00:42:46.860 --> 00:42:48.919 onto the ketone. You found this.
NOTE Confidence: 0.8315405
00:42:48.919 --> 00:42:49.948 Finally this image.
NOTE Confidence: 0.8315405
00:42:49.950 --> 00:42:52.116 We then do a bond formation
NOTE Confidence: 0.8315405
00:42:52.116 --> 00:42:54.254 deprotect the Bach route to get
NOTE Confidence: 0.8315405
00:42:54.254 --> 00:42:56.126 to this compound on the left,
NOTE Confidence: 0.8315405
00:42:56.130 --> 00:42:59.698 we isolate this as as it’s TFA salt.
NOTE Confidence: 0.8315405
00:42:59.700 --> 00:43:01.730 But if you neutralize this,
NOTE Confidence: 0.8315405
00:43:01.730 --> 00:43:03.755 it’s snapshot and so this
NOTE Confidence: 0.8315405
00:43:03.755 --> 00:43:05.375 carbon attacks this ketone,
NOTE Confidence: 0.8315405
00:43:05.380 --> 00:43:08.050 you lose water, any formatting.
NOTE Confidence: 0.8315405
00:43:08.050 --> 00:43:08.830 And.
NOTE Confidence: 0.8187508
00:43:10.920 --> 00:43:12.838 The assay that we use, that’s ’cause
NOTE Confidence: 0.8187508
00:43:12.838 --> 00:43:15.400 it’s nice to give us a lot of detail.
NOTE Confidence: 0.8187508
00:43:15.400 --> 00:43:17.080 It’s inexpensive, it’s fast, is is.
NOTE Confidence: 0.808242
00:43:19.250 --> 00:43:20.934 Taking linearized plasmid DNA
NOTE Confidence: 0.808242
00:43:20.934 --> 00:43:22.618 incubating with the molecule,
NOTE Confidence: 0.808242
00:43:22.620 --> 00:43:24.300 running a denaturing gel,
NOTE Confidence: 0.808242
00:43:24.300 --> 00:43:26.820 and basically if you look at
NOTE Confidence: 0.808242
00:43:26.905 --> 00:43:28.930 the right hand lanes here,
NOTE Confidence: 0.808242
00:43:28.930 --> 00:43:31.597 you see these streaks on the gel
NOTE Confidence: 0.808242
00:43:31.597 --> 00:43:34.742 going down to about 100 animal or what
NOTE Confidence: 0.808242
00:43:34.742 --> 00:43:38.229 that tells us is that at 100 animal
NOTE Confidence: 0.808242
00:43:38.229 --> 00:43:40.719 or concentration of this compound,
NOTE Confidence: 0.808242
00:43:40.720 --> 00:43:41.983 we’re getting extensive
NOTE Confidence: 0.808242
00:43:41.983 --> 00:43:43.667 degradation of the DNA.
NOTE Confidence: 0.808242
00:43:43.670 --> 00:43:46.190 These are smaller fragments that
NOTE Confidence: 0.808242
00:43:46.190 --> 00:43:49.140 have higher mobility on the gel.
NOTE Confidence: 0.808242
00:43:49.140 --> 00:43:52.436 And so that was very exciting to us.
And you know, we hypothesized again that it was this nucleotide addition to the cyclopropane that was leading to this degradation of the DNA. And so to probe that in a little bit more detail, we made a couple of control compounds. So the first one. Was this dimeric structure up top here and so the hypothesis is that if this is alkylating DNA, perhaps we can induce two fold alkylation and perhaps we can then detect and interstrand crosslink?
And when you incubate with that compound, indeed you can see down here Crosslink Band. This corresponds to our positive control for crosslinking cisplatin.

And then the other thing we did was we made a negative control where we took that cyclopropane and converted it to the gem dimethyl substituent. The hypothesis being if the cyclopropane is truly involved, this compound should be inactive and going up to half millimolar. We don’t detect any damage in our assay and so without characterizing the product without even having isolated the natural products,
we were able to sort of formulate this proposal for how these things might be. Might be alkylating DNA. And. We are, you know, we sort of at that point. Got stocks so that was around 2018 when we had identified this. You know, this DNA reactive substructure in the molecule. We knew that it was incomplete. there were other functional groups, other rings and things with in Cali bactine. But we didn’t know what they were. And as I mentioned in the beginning,
the classical isolation approach is not very successful in this context, and so we were stuck. Until this paper came out, and so this is also from the Oswald Group. They did a beautiful experiment where they took the collie, backed in producing bacteria, grew them up in liquid media, added exogeneous DNA, isolated that DNA following incubation, and ran a denaturing gel and the point is that they observed interstrand crosslinks in that DNA that was exposed to these bacteria OK and so.
I have a note here at the bottom to remind you know if you’re paying attention.

The original female phenotype was double strand breaks.

Now I’m talking about cross links. Those two lesions are intimately related, and I’ll talk about that at the end if there’s time.

But basically we’re very excited about this paper because you can imagine that Kohli bactine is entrained in that crosslink, right?

You know, if that’s what’s causing the crossing at that point wasn’t completely certain that either,
but. Assuming that it is all we have to do, all we have to do is isolate that crosslink can characterize it. And so at this point in time, you know. We had a very good understanding of what went in of the amino acids that went into the pathway and where they ended up. Following sort of offloading of the biosynthetic products, and so for example, it was known through work that Jason did very early on, and then others that this amino cyclopropane comes from methionine,
and these thiazole rings derived from cysteine OK.

And So what?

This allowed us to do is conduct essentially isotope labeling experiments where we generated auxotrophic strains, either deficient in methionine or cysteine biosynthesis, and then supplemented those cultures with C13 labeled amino acid OK.

And so we can take the wild type strain the oxygen riffs with their amino acids incubate.

Here we’re using linearized puck 19 DNA. We can run a gel to verify.
that we got crosslinking, and then we can try and isolate that cross link and characterize it, and one of the things that’s worth pointing out is that to do these assays were talking about 250 microliters were talking about 250 microliters of culture versus 2000 liters. You know using the old? The sort of the old fashioned method, and so to give you an idea of what the data looks like and why we do this isotope labeling. I’ll show you this slide. So for example, we can spot these ions that I’ve marked in colored boxes here,
and the top chromatogram is the wild type strain. And what you can see in the Sistine Auxotroph the middle graph is that those ions are shifted by three units, and so that’s very useful to us because it tells us two things. One is that that Ion is probably contains Poly back in or the vestiges of Cali Bactine and then two. It contains one thiazole residue, there was one cysteine incorporated into that unit. And we can play the same game with the methionine auxotroph.
00:49:19.600 --> 00:49:22.129 So here we get a shift by plus four.
NOTE Confidence: 0.8377484

00:49:22.130 --> 00:49:24.118 So that tells us there’s 11 amino
NOTE Confidence: 0.8377484

00:49:24.118 --> 00:49:25.565 cyclopropane and tells us it’s
NOTE Confidence: 0.8377484

00:49:25.565 --> 00:49:26.905 also related to Cali Bactine.
NOTE Confidence: 0.8377484

00:49:26.910 --> 00:49:30.870 OK, so this was the initial work that we did.
NOTE Confidence: 0.8377484

00:49:30.870 --> 00:49:34.070 We had to carry out a lot more
NOTE Confidence: 0.8377484

00:49:34.070 --> 00:49:37.545 labeling in order to get the full
NOTE Confidence: 0.8377484

00:49:37.545 --> 00:49:40.615 structure assignment and So what we
NOTE Confidence: 0.8377484

00:49:40.615 --> 00:49:43.815 did was we generated a series of we
NOTE Confidence: 0.8377484

00:49:43.815 --> 00:49:46.450 had our cysteine and methionine auxotroph.
NOTE Confidence: 0.8377484

00:49:46.450 --> 00:49:49.000 We generated steering and glaci Knox
NOTE Confidence: 0.8377484

00:49:49.000 --> 00:49:51.694 Atros because those are also incorporated
NOTE Confidence: 0.8377484

00:49:51.694 --> 00:49:54.454 into the into the natural product.
NOTE Confidence: 0.8377484

00:49:54.460 --> 00:49:58.060 And then we also did Universal labeling C
NOTE Confidence: 0.8377484

00:49:58.060 --> 00:50:01.430 13 labeling with glucose an 15 labeling.
NOTE Confidence: 0.8377484

00:50:01.430 --> 00:50:02.864 With ammonium chloride.
And we can run the same experiment where we incubate with the DNA, isolate the crosslink, digest it, analyze it by 10MM S and we can then see different shifts in those ions. This data turned out to be very powerful for us, because without isolating the compound we can glean an incredible amount of insight into the molecule structure. So from the glucose labeling, we get a shift by 37 units.
That tells us, of course, it has 37 carbons. Ammonia shifts by 8 units, we have eight nitrogens we can see that in the methionine auxotroph, and I'm talking about a higher molecular weight ion here. At the top we get a shift by 8 carbons and so that told us that we had two of these cyclopropane residues or what was left of them. Two thiazole rings based on A6 and so we can basically tease out a lot of structural data to sort of
00:51:09.138 --> 00:51:12.097 see what pieces are need to be put together here to make the molecule.

00:51:14.240 --> 00:51:18.152 And so at any rate we found this higher molecular weight ion at 956.

00:51:21.030 --> 00:51:22.298 Using all that data, we were able to fit it to this structure here and so it contains one adenine residue and have explicitly drawn the adenine without connectivity to the base, because at the time that we did this, we couldn’t specify where it was bonded to adenine.

00:51:41.170 --> 00:51:44.047 We now know that that’s in three, but had one adenine on the right hand side.

00:51:47.750 --> 00:51:50.620 You have a cyclopropane that’s still intact,
And then you’ve got the rest of the core molecule sort of linking it together, and so it’s almost C2 symmetric, it’s a hetero dimer. It’s not quite C2 symmetric. If you look carefully at these thiazole rings they have different appendages in different connectivity, but it’s very close OK, and this structure fit RMS data within one PPM, so we’re very excited about that. And so if that is simply a mono adenine addict and we’re getting icy, else, presumably there’s a.
NOTE Confidence: 0.7616281
00:52:22.380 --> 00:52:24.960 dinucleotide add up and we went,
NOTE Confidence: 0.7616281
00:52:24.960 --> 00:52:29.260 and we were able to find the dyad an addict.
NOTE Confidence: 0.7616281
00:52:29.260 --> 00:52:33.130 OK, and this fits, fits within 1/2 PPM error.
NOTE Confidence: 0.7616281
00:52:33.130 --> 00:52:35.280 OK, and so working backwards,
NOTE Confidence: 0.7616281
00:52:35.280 --> 00:52:38.720 if that’s the dyad, and in an act,
NOTE Confidence: 0.7616281
00:52:38.720 --> 00:52:41.730 then this is the structure of Kohli,
NOTE Confidence: 0.7616281
00:52:41.730 --> 00:52:44.140 bactine on the bottom here.
NOTE Confidence: 0.7616281
00:52:44.140 --> 00:52:47.899 OK, and so we’ve got two cyclopropane’s.
NOTE Confidence: 0.7616281
00:52:47.900 --> 00:52:52.366 And in the middle we have this
NOTE Confidence: 0.7616281
00:52:52.366 --> 00:52:54.770 1/2 dicarbonyl residue OK.
NOTE Confidence: 0.7616281
00:52:54.770 --> 00:52:56.402 There’s a detail here
NOTE Confidence: 0.7616281
00:52:56.402 --> 00:52:58.034 which is worth mentioning,
NOTE Confidence: 0.7616281
00:52:58.040 --> 00:53:01.712 which is that this is this kind of compound.
NOTE Confidence: 0.7616281
00:53:01.720 --> 00:53:04.672 On the bottom is what we characterized what
NOTE Confidence: 0.7616281
00:53:04.672 --> 00:53:07.858 we expect based on the biosynthetic pathway.
NOTE Confidence: 0.7616281
00:53:07.860 --> 00:53:11.124 Is the self amino ketone at the top,
NOTE Confidence: 0.7616281
00:53:11.130 --> 00:53:13.594 but we’ve done work that shown that
NOTE Confidence: 0.7616281
00:53:13.594 --> 00:53:16.116 this thing is unstable towards aerobic
NOTE Confidence: 0.7616281
00:53:16.116 --> 00:53:18.894 oxidation to an Alpha keto imine,
NOTE Confidence: 0.7616281
00:53:18.900 --> 00:53:21.959 and then hydrolysis 212 die ketone and
NOTE Confidence: 0.7616281
00:53:21.959 --> 00:53:24.697 so working under air on the bench.
NOTE Confidence: 0.7616281
00:53:24.700 --> 00:53:27.171 This is this is the compound that
NOTE Confidence: 0.7616281
00:53:27.171 --> 00:53:29.490 you would have expected to get.
NOTE Confidence: 0.7616281
00:53:29.490 --> 00:53:31.620 And still so no ones isolated
NOTE Confidence: 0.7616281
00:53:31.620 --> 00:53:33.040 calling back in yet.
NOTE Confidence: 0.7616281
00:53:33.040 --> 00:53:35.170 And so how do you prove
NOTE Confidence: 0.7616281
00:53:35.170 --> 00:53:36.235 the structure assignment?
NOTE Confidence: 0.7616281
00:53:36.240 --> 00:53:39.426 We can go back and try and make it,
NOTE Confidence: 0.7616281
00:53:39.430 --> 00:53:42.286 and so we spent some time developing a
NOTE Confidence: 0.7616281
00:53:42.286 --> 00:53:44.757 synthesis of the molecule and it was.
NOTE Confidence: 0.7616281
00:53:44.760 --> 00:53:46.890 It was not straightforward because of
00:53:46.890 --> 00:53:50.210 its instability, but we could make it.

00:53:50.210 --> 00:53:54.107 And then we can do an LCMS coinjection and

00:53:54.107 --> 00:53:58.207 we see that has the same retention time.

00:53:58.210 --> 00:54:00.821 It has the same tandem Ms as

00:54:00.821 --> 00:54:03.670 the as the natural material,

00:54:03.670 --> 00:54:06.645 and then finally we did a crosslinking

00:54:06.645 --> 00:54:09.772 assay where we basically ran that same

00:54:09.772 --> 00:54:13.230 experiment that we ran with the bacteria,

00:54:13.230 --> 00:54:15.526 except replace the bacteria

00:54:15.526 --> 00:54:17.248 with our compound.

00:54:17.250 --> 00:54:19.464 And so this thing will crosslink

00:54:19.464 --> 00:54:21.676 add up to about, you know,

00:54:21.676 --> 00:54:23.516 down to about 500 nanomolar.

00:54:23.520 --> 00:54:26.200 And then we can do the tandem Ms

00:54:26.200 --> 00:54:28.320 analysis of those cross links.
And so let me explain what’s on this slide.

So when we do the bacterial experiment

where we treat the DNA with the bacteria,

we can isolate the crosslink.

You then run tandem Ms,

you get a whole list of ions,

primary and secondary and tertiary ions

that you see from those crosslinks and so.

You know the argument is.

If we’re making the same molecule

Are synthetic compound oughta interact

with DNA in the same way and it ought to

blow apart in a mass spec in the same way?

And So this plot shows on the

X axis or all of the ions that
we found in the tenant and Ms of the bacteria derived crosslinks, we see all of those ions with our synthetic material. And the Y axis is simply the experimental minus theoretical error for those ions using this synthetic material. And so the point is we get all the same ions that we get when we use the bacteria. They’re all within with the exception of 1 within two PPM OK, and so we don’t have an NMR of Cali bactine to compare to. But we can say that structure that we made interacts with DNA.
00:55:46.790 --> 00:55:49.142 It crosslinks DNA and then it blows
NOTE Confidence: 0.7819776
00:55:49.142 --> 00:55:51.210 apart in attend imeson exactly.
NOTE Confidence: 0.7819776
00:55:51.210 --> 00:55:53.031 In indistinguishable fashion.
NOTE Confidence: 0.7819776
00:55:53.031 --> 00:55:56.066 And So what about this?
NOTE Confidence: 0.7819776
00:55:56.070 --> 00:55:58.368 I see LDS be, you know,
NOTE Confidence: 0.7819776
00:55:58.370 --> 00:55:59.104 apparent contradiction,
NOTE Confidence: 0.7819776
00:55:59.104 --> 00:56:02.040 so there's been a lot of debate in
NOTE Confidence: 0.7819776
00:56:02.105 --> 00:56:04.130 the literature between you know,
NOTE Confidence: 0.7819776
00:56:04.130 --> 00:56:06.370 debating the mechanism of action
NOTE Confidence: 0.7819776
00:56:06.370 --> 00:56:08.610 because Oswald had originally observed
NOTE Confidence: 0.7819776
00:56:08.677 --> 00:56:11.015 DNA double strand breaks using a comet
NOTE Confidence: 0.7819776
00:56:11.015 --> 00:56:13.347 assay and then came along and said,
NOTE Confidence: 0.7819776
00:56:13.350 --> 00:56:15.270 no wait, it’s cross links,
NOTE Confidence: 0.7819776
00:56:15.270 --> 00:56:17.974 and for any of you that are familiar
NOTE Confidence: 0.7819776
00:56:17.974 --> 00:56:19.879 with these repair pathways,
NOTE Confidence: 0.7819776
00:56:19.880 --> 00:56:22.178 you know that these two phenotypes
are intimately linked, right? And so.

When you start to repair an ICL, you actually form a DSP that leads to activation of HR and so you’re going to see gamma, H2, X. You’re going to see streaking in your comment essay, and so the two phenotypes are entirely consistent.

And we actually identified another pathway, which is just a spontaneous pathway. It’s well known in the old sort of Gina toxin literature that N3 adenine addicts are unstable towards depurination.
And if we run our crosslinking assay, we sort of. We modify the assay to be able to sort of get at this data, but this is the conclusion is essentially that these Icl’s undergo a slow, deep urination. And then there’s a second elimination of the phosphate that occurs to lead to a single strand break. And you can imagine then you know, in tandem with the repair pathways and also other alkylation lesions. Eventually you’re going to get those single strand breaks close enough to each other to get a double strand break.
And so this brings us to where we're at in the project, and so.

What we're currently doing is working with this molecule on the bottom here. And this is not Kohli backed in itself. It is a analog of Kohli Bakhtin. The differences are highlighted in green, and so the dye ketonen Kohli backed in on the top here is very unstable. You can't work with this compound, you know Suficicent it would be, you know Suficicent asked to try and use this in a series of essays to examine it. Sort of cellular activity. And so we made what we call the
Dez di ketone analog on the bottom and working with Christian Jobin at the University of Florida. Christians been basically taking this compound. Through all of the essays that have been run by Oswald and Box Tone Cleavers and others looking at the genotoxic phenotype of the bacteria and seeing if the molecule recapitulates it and up until now, it seems too so we see we get activation again.

What he’s doing right now is basically looking to see if this induces the same
00:58:50.123 --> 00:58:52.343 type of mutational signature that one
00:58:52.343 --> 00:58:54.823 gets with the bacteria and that will be,
00:58:54.830 --> 00:58:56.888 you know, sort of the end.
00:58:56.890 --> 00:58:57.793 The end point.
00:58:57.793 --> 00:58:59.298 Hopefully you know it will
00:58:59.298 --> 00:59:01.019 be a positive result,
00:59:01.020 --> 00:59:03.694 but whatever it is that will be
00:59:03.694 --> 00:59:06.529 sort of the endpoint for this.
00:59:06.530 --> 00:59:08.420 And so I just acknowledge all
00:59:08.420 --> 00:59:10.330 the people that did the work.
00:59:10.330 --> 00:59:11.915 I'll just go through this
00:59:11.915 --> 00:59:13.183 quickly to save time.
00:59:13.190 --> 00:59:15.086 But this is my collaborator Jason.
00:59:15.090 --> 00:59:16.670 Many of you know him.
00:59:16.670 --> 00:59:18.590 Many people from my group contributed
to this project over the years,
NOTE Confidence: 0.8687447
acknowledged the NCI and Yale for funding.
NOTE Confidence: 0.8687447
Thanks again for the invitation
NOTE Confidence: 0.8687447
and I’m happy to stay on and
take any questions you have.
NOTE Confidence: 0.8345129
Thank you Seth, very interesting.
NOTE Confidence: 0.8345129
It makes me appreciate
NOTE Confidence: 0.8345129
that I’m a microbiologist.
NOTE Confidence: 0.8345129
It’s not nowhere near as hard
NOTE Confidence: 0.8345129
as being a chemist, I think.
NOTE Confidence: 0.8345129
Are there other questions for process?
NOTE Confidence: 0.81077635
I want question of course,
NOTE Confidence: 0.81077635
DNA damaging agents can cause cancer,
NOTE Confidence: 0.81077635
but there also used to treat cancer.
NOTE Confidence: 0.81077635
Is there any thought? Is there
NOTE Confidence: 0.81077635
possibility of using these compounds
01:00:01.380 --> 01:00:02.748 therapeutically? Absolutely yeah, that’s
01:00:02.750 --> 01:00:04.450 something we’re very excited about,
01:00:04.450 --> 01:00:06.496 so I didn’t get into it.
01:00:06.500 --> 01:00:08.614 You know, one of the challenges that
01:00:08.614 --> 01:00:11.608 we in my group is always the chemistry.
01:00:11.610 --> 01:00:13.500 You know, the chemistry work is
01:00:13.500 --> 01:00:15.448 a general chemistry to make these
01:00:15.448 --> 01:00:17.618 molecules is very robust and we started
01:00:17.618 --> 01:00:19.799 to characterize them with Ranjeet,
01:00:19.800 --> 01:00:21.505 Bindra slab and he’s found
01:00:21.505 --> 01:00:23.210 that in bracket two mutants,
01:00:23.210 --> 01:00:25.926 these things are hyperactive and so that’s
01:00:25.926 --> 01:00:28.030 the immediate direction we’re going in.
01:00:28.030 --> 01:00:30.748 Long term, we’re looking to see if we can,
01:00:30.750 --> 01:00:32.616 you know, optimize the properties of

these molecules a little bit more.

You know it’s more than just another crosslinker because I didn’t get into it,

but there’s a mechanism by which we can gauge the activity of the molecule and potentially target it,

and so there’s a lot.

Yeah, there’s a lot that we can do,

that’s sort of the phase we’re entering into with the project traffic we are after

1:00 o’clock, so if people have other questions for Seth,

just email him I’m sure. Be happy too.

Talking thank both speakers for really stimulating talk today. Thank
01:01:03.600 --> 01:01:05.049 you thanks everyone.