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, and 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 glioma or glioblastoma, still is a relatively rare disease with only three point 1 patients for each 100,000 people.

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 present
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
has shown to improve survival so far is this alkylation alkylation agent that is more than 20 years old. So this is the most dolomite and in controllers here is saying that the Missouri might improves both work for survival and overall survival, but even the experimental arm in the pivotal trial, which was published in 2005. Survival remained only 15 months for again, newly diagnosed disease and further analysis of this data has shown that this survivor benefit is mostly driven by tumors that have this afternoon. 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 therapiest.
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 biome’s 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 algae blooms, which is more penalty? Q coalition ideate mutation Sir 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
to fication 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

NOTE Confidence: 0.7912263

you see also a lot of these patients

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with arousing in the T3 pathway leading to.

NOTE Confidence: 0.7912263

Abnormalities in senescence and a pop

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ptosis and a lot of these patients

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having a normality’s in cell cycle control.

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But then when we put all of these

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mutations have a track record of

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being drug and what you can see is

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unfortunately each of these mutations

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is actually very where we’re not

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being very good at identifying

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drugs for those specific phenotypes

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we heavily rely on basket trials.

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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 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
potential target mutations are actually gone.

We’re seeing some passengers here. But the reality that’s what’s driving this tumor now is actually probably about Melanie’s at the OBGYN attic level, and you can imagine that if at this point in time of her disease we work to enroll her in a clinical trial. Most patients do not have another brain surgery to have another sequence, so you go to archive tissue and we would have selected her for trials that probably were irrelevant for her at this point in time. 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. This 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. Therapies, if anything,
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.
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 could 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,
00:15:32.015 --> 00:15:34.145 both newly diagnose and recurrent disease. 
NOTE Confidence: 0.8497241
00:15:37.040 --> 00:15:39.238 So we’re not giving up on immunotherapy, 
NOTE Confidence: 0.8497241
00:15:39.240 --> 00:15:42.230 so I think our task now is to try to 
NOTE Confidence: 0.8497241
00:15:42.320 --> 00:15:45.148 send what is that about the brain? 
NOTE Confidence: 0.8497241
00:15:45.150 --> 00:15:48.146 That in spite of PDL one expression 
NOTE Confidence: 0.8497241
00:15:48.146 --> 00:15:50.865 we’re not seeing any help from Anti 
NOTE Confidence: 0.8497241
00:15:50.865 --> 00:15:53.818 PD one or anti PDL one therapies and 
NOTE Confidence: 0.8497241
00:15:53.818 --> 00:15:57.178 I think for for this question I think 
NOTE Confidence: 0.8497241
00:15:57.180 --> 00:16:00.375 it is great to have a helping hand of 
NOTE Confidence: 0.8497241
00:16:00.375 --> 00:16:02.993 people that study the CNS immunology 
NOTE Confidence: 0.8497241
00:16:02.993 --> 00:16:06.834 and in this project what we did is to 
NOTE Confidence: 0.8497241
00:16:06.834 --> 00:16:09.204 partner with Doctor David Hoffer and 
NOTE Confidence: 0.8497241
00:16:09.210 --> 00:16:12.266 I’ll also Liliana Luca to look at how 
NOTE Confidence: 0.8497241
00:16:12.266 --> 00:16:15.646 can we actually come up with better. 
NOTE Confidence: 0.8497241
00:16:15.650 --> 00:16:18.070 Image checkpoint inhibition that is 
NOTE Confidence: 0.8497241
00:16:18.070 --> 00:16:20.981 relevant for for this Mike environment
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.

Also, to understand a little
bit more of the immune system, we need good models that are immunocompetent and one of the. A richness of our environment here is Doctor City chains work producing these jam models of stomas where he can pretty much produce avatars for all of these phenotypes that I just showed you, and one of the ideas here is to see how these different phenotypes respond to these different immunotherapy’s. So this is very exciting data which again illustrates how we can concomitantly to the development in the clinic to also try to understand.
Are these treatments in parallel in the lab?

Now another barrier for.

For the development of effective 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
potentially intervene in this was discovered by an item here at go, where she’s looking at the role of this little Robo one, signaling which seems to attract and polarize. Save Microfridge is in in the brain microenvironment and Livingstone my confirming. And when she did experiments to knock down, slid to, or to block this pathway, she achieved better immune responses and inflammation of anti PD one. She had a really significant improvement in survival or in this tumor bearing mice. So the idea here is now to generate
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, which is the so-called race essay which is a disrupted analysis of cell phenotype extremes where he uses the cell migration as a surrogate marker of tumor aggressiveness and then you can test the multiple 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. 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
multi 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, 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. Kevin versus Kevin Beans loosely, and neither will all understand importance.
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, 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.
00:29:37.300 --> 00:29:39.544 Although most of these drugs would
NOTE Confidence: 0.71716356
00:29:39.544 --> 00:29:42.199 actually be again in rare phenotypes,
NOTE Confidence: 0.71716356
00:29:42.200 --> 00:29:44.240 because I think those are,
NOTE Confidence: 0.71716356
00:29:44.240 --> 00:29:46.688 we still need to select them
NOTE Confidence: 0.71716356
00:29:46.688 --> 00:29:48.320 by those specific mutations.
NOTE Confidence: 0.71716356
00:29:48.320 --> 00:29:50.768 The problem of copy number remains
NOTE Confidence: 0.71716356
00:29:50.768 --> 00:29:52.400 regardless of flipping connectors.
NOTE Confidence: 0.71716356
00:29:52.400 --> 00:29:55.256 I don’t think blood brain barrier penetration
NOTE Confidence: 0.71716356
00:29:55.256 --> 00:29:58.152 was the reason why we couldn’t target
NOTE Confidence: 0.71716356
00:29:58.152 --> 00:30:00.558 EGFR amplification or Pete and loss.
NOTE Confidence: 0.71716356
00:30:00.560 --> 00:30:03.409 I think that is a different question,
NOTE Confidence: 0.71716356
00:30:03.410 --> 00:30:06.308 but if we are to even answer
NOTE Confidence: 0.71716356
00:30:06.308 --> 00:30:08.130 those we still need.
NOTE Confidence: 0.71716356
00:30:08.130 --> 00:30:09.038 This kind of approach,
NOTE Confidence: 0.71716356
00:30:09.038 --> 00:30:10.999 ’cause it makes our life so much easier.
NOTE Confidence: 0.8856998
00:30:13.660 --> 00:30:15.550 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.
Changes.

So there’s a whole line of research trying to then understand this and more canals are interested 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.

We have to move on to the second speaker.

In our second stewartii climb down from the Hill from Science Hill, is Seth hairs on who’s the Milton
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.

Comment for attending the lecture.

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 will be a story for another day.

And so right. So today I'll talk
about a project that’s been ongoing in my group for about 6 years. And we’ve been looking to understand the molecular basis of a carcinogen carcinogenic phenotype that was observed from certain gut bacteria so. I’ll go through sort of the sequence of events to kind of outline sequence of discoveries to outline the problem, and so in 2006 this was the paper that set off a lot of interest in this area. Eric Oswald and coworkers identified certain strains of commensal and pathogenic E coli that had a biosynthetic gene cluster known as the CLB cluster.
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 was 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 then 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.
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 you know,
had done a series of very nice and you know,
robust control experiments to establish
that this genotoxic phenotype.
Is due to the final biosynthetic
product 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
00:38:52.155 --> 00:38:54.560 HPLC and then we typically do.

00:38:54.560 --> 00:38:57.020 It’s known as activity guided fractionation,

00:38:57.020 --> 00:39:01.940 where you’re essentially testing each of

00:39:01.940 --> 00:39:04.978 these fractions for a particular phenotype.

00:39:04.978 --> 00:39:07.916 And then you keep purifying and testing

00:39:07.916 --> 00:39:10.550 and purifying testing until you get to

00:39:10.550 --> 00:39:12.530 a single compound and you characterize it.

00:39:12.530 --> 00:39:15.060 The problem is that this

00:39:15.060 --> 00:39:16.130 approach does not work for Kohli.

00:39:16.130 --> 00:39:23.612 Bakhtin, OK,

00:39:23.612 --> 00:39:27.296 so the molecule is very unstable.

00:39:27.300 --> 00:39:32.016 And what we find is that because of the.

00:39:32.020 --> 00:39:32.880 You know,
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 to.
Give you an example of how challenging this is.
This is not work from our own laboratory. This is a group at at Berkeley and scripts.
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 to the genotoxic phenotype. 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,
we’ve been collaborating with Jason Crawford.

Jason is one of the leaders in understanding Kohli, backed in biosynthesis.

And So what we’ve been doing is really taking knowledge from the biosynthetic pathway and trying to infer what types of substructures might be within Kohli. With DNA and so one of the sort of models that came out of these biosynthetic studies is that you have these fully linear products offloaded from the PKS assembly line. There’s a searing protease that
00:41:28.970 --> 00:41:32.179 removes this residue and blew this ACL.

NOTE Confidence: 0.8315405

00:41:32.180 --> 00:41:34.230 Asparagine residue.

NOTE Confidence: 0.8315405

00:41:34.230 --> 00:41:36.250 That generates a primary amine,

NOTE Confidence: 0.8315405

00:41:36.250 --> 00:41:38.742 and once you format that can start

NOTE Confidence: 0.8315405

00:41:38.742 --> 00:41:41.666 to wrap up and ultimately lead to

NOTE Confidence: 0.8315405

00:41:41.666 --> 00:41:44.324 this compound on the bottom here,

NOTE Confidence: 0.8315405

00:41:44.330 --> 00:41:46.784 which has a cyclopropane ring in

NOTE Confidence: 0.8315405

00:41:46.784 --> 00:41:49.578 conjugation with with it with the Alpha,

NOTE Confidence: 0.8315405

00:41:49.580 --> 00:41:51.575 beta unsaturated imming and for

NOTE Confidence: 0.8315405

00:41:51.575 --> 00:41:54.042 those in the audience that have

NOTE Confidence: 0.8315405

00:41:54.042 --> 00:41:55.638 worked with Gina toxins,

NOTE Confidence: 0.8315405

00:41:55.640 --> 00:41:57.660 you know that these electrophilic

NOTE Confidence: 0.8315405

00:41:57.660 --> 00:41:59.276 cyclopropane’s are not uncommon.

NOTE Confidence: 0.8315405

00:41:59.280 --> 00:42:02.264 This is a sort of a pharmacophore that’s

NOTE Confidence: 0.8315405

00:42:02.264 --> 00:42:05.460 found in a variety of genotoxic natural.

NOTE Confidence: 0.8315405

66
Products and so this was,

you know,

sort of very logically following

from that type of precedent.

The problem is that the problem is this.

No one had isolated these imines.

No one had any spectroscopic data on them.

All we had was this.

This kind of this 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

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
concentrate this down from dilute acid,

we can get this.

Carbon and nitrogen to condense onto the ketone. You found this.

Finally this image.

We then do a bond formation deprotect the Bach route to get to this compound on the left,

we isolate this as as it’s TFA salt. But if you neutralize this,

it’s snapshot and so this carbon attacks this ketone,

you lose water, any formatting. And.

The assay that we use, that’s ’cause
it’s nice to give us a lot of detail.

It’s inexpensive, it’s fast, is is.

Taking linearized plasmid DNA incubating with the molecule, running a denaturing gel, and basically if you look at the right hand lanes here, you see these streaks on the gel going down to about 100 animal or concentration of this compound, that tells us is that at 100 animal or concentration of this compound, we’re getting extensive degradation of the DNA. These are smaller fragments that have higher mobility on the gel. 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. We are, you know, we sort of at that point. Got stocks so that was around 2018 when we had identified this. We knew that it was incomplete. In other words, 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.
00:46:18.950 --> 00:46:21.970 I have a note here at the bottom to remind me you know if you’re paying attention.

00:46:22.044 --> 00:46:24.844 The original female phenotype was double strand breaks.

00:46:24.850 --> 00:46:26.238 Now I’m talking about cross links.

00:46:26.238 --> 00:46:27.626 Those two lesions are intimately related, and I’ll talk about that at the end if there’s time.

00:46:27.630 --> 00:46:29.706 But basically we’re very excited about this paper because you can imagine that Kohli bactine is entrained in that crosslink,

00:46:29.710 --> 00:46:32.139 Those two lesions are intimately related,

00:46:32.140 --> 00:46:34.162 and I’ll talk about that at the end if there’s time.

00:46:34.162 --> 00:46:35.950 But basically we’re very excited about this paper because you can imagine that Kohli bactine is entrained in that crosslink,

00:46:35.950 --> 00:46:37.996 if that’s what’s causing the crossing at that point wasn’t completely certain that either,

00:46:37.996 --> 00:46:40.461 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. 90% of the biosynthetic pathway had been mapped out. 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.

And so what?

This allowed us to 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.

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

Here we’re using linearized puck 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 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,
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.
So here we get a shift by plus four. So that tells us there’s 11 amino cyclopropane and tells us it’s also related to Cali Bactine. OK, so this was the initial work that we did. We had to carry out a lot more labeling in order to get the full structure assignment and So what we did was we generated a series of we had our cysteine and methionine auxotroph. Atros because those are also incorporated into the natural product. And then we also did Universal labeling C 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. And this data turned out to be very powerful for us, because without isolating the compound without getting any spectroscopic data, 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, that 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 two of these cyclopropane residues or what was left of them. Two thiazole rings based on A6 Two thiazole rings based on A6 if you get the idea, and so we can basically tease out a lot of structural data to sort of
see what pieces are needed to be put together here to make the molecule. And so at any rate we found this higher molecular weight ion at 956. 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. We now know that that’s in three, but had one adenine on the right hand side. You have a cyclopropane that’s still intact,
00:51:50.620 --> 00:51:50.909 OK.
NOTE Confidence: 0.7990484
00:51:50.909 --> 00:51:53.510 And then you’ve got the rest of the core
NOTE Confidence: 0.7990484
00:51:53.583 --> 00:51:56.019 molecule sort of linking it together,
NOTE Confidence: 0.7990484
00:51:56.020 --> 00:51:58.225 and so it’s it’s almost C2 symmetric,
NOTE Confidence: 0.7990484
00:51:58.230 --> 00:51:59.820 it’s it’s a hetero dimer.
NOTE Confidence: 0.7990484
00:51:59.820 --> 00:52:01.400 It’s not quite C2 symmetric.
NOTE Confidence: 0.7990484
00:52:01.400 --> 00:52:02.924 If you look carefully at these
NOTE Confidence: 0.7990484
00:52:02.924 --> 00:52:04.422 thiazole rings they have different
NOTE Confidence: 0.7990484
00:52:04.422 --> 00:52:06.158 appendages in different connectivity,
NOTE Confidence: 0.7990484
00:52:06.160 --> 00:52:07.740 but it’s very close OK,
NOTE Confidence: 0.7990484
00:52:07.740 --> 00:52:09.325 and this structure fit RMS
NOTE Confidence: 0.7990484
00:52:09.325 --> 00:52:10.593 data within one PPM,
NOTE Confidence: 0.7990484
00:52:10.600 --> 00:52:12.496 so we’re very excited about that.
NOTE Confidence: 0.7616281
00:52:14.640 --> 00:52:17.848 And so if that is simply a mono
NOTE Confidence: 0.7616281
00:52:17.848 --> 00:52:20.657 adenine addict and we’re getting icy,
NOTE Confidence: 0.7616281
00:52:20.660 --> 00:52:22.380 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
84
Is the self amino ketone at the top, but we've done work that shown that this thing is unstable towards aerobic oxidation to an Alpha keto imine, and then hydrolysis die ketone and working under air on the bench. This is this is the compound that you would have expected to get. And still so no ones isolated calling back in yet. And so how do you prove the structure assignment? We can go back and try and make it, and so we spent some time developing a synthesis of the molecule and it was. It was not straightforward because of
its instability, but we could make it.
And then we can do an LCMS coinjection and we see that has the same retention time.
It has the same tandem Ms as the as the natural material,
and then finally we did a crosslinking assay where we basically ran that same experiment that we ran with the bacteria,
except replace the bacteria with our compound.
And so this thing will crosslink add up to about, you know,
down to about 500 nanomolar.
And then we can do the tandem Ms 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, getting a whole list of ions, primary and secondary and tertiary ions that you see from those crosslinks and so.

The argument is: If we’re making the same molecule that the bugs are making, are synthetic compound oughta interact with DNA in the same way and blow apart in a mass spec in the same way? And so what 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.
It crosslinks DNA and then it blows apart in indistinguishable fashion. So what about this? I see LDS be, you know, apparent contradiction, so there’s been a lot of debate in the literature between you know, debating the mechanism of action because Oswald had originally observed DNA double strand breaks using a comet assay and then came along and said, no wait, it’s cross links, and for any of you that are familiar with these repair pathways, you know that these two phenotypes...
00:56:22.178 --> 00:56:24.413 are intimately linked, right? And so.
00:56:24.413 --> 00:56:26.940 When you start to repair an ICL,
00:56:26.940 --> 00:56:29.046 you actually form a DSP that
00:56:29.046 --> 00:56:31.457 leads to activation of HR and so
00:56:31.457 --> 00:56:33.662 you’re going to see gamma, H2, X.
00:56:33.662 --> 00:56:35.292 You’re going to see streaking
00:56:35.292 --> 00:56:36.710 in your comment essay,
00:56:36.710 --> 00:56:38.885 and so the two phenotypes
00:56:38.885 --> 00:56:40.190 are entirely consistent.
00:56:40.190 --> 00:56:42.896 And we actually identified another pathway,
00:56:42.900 --> 00:56:45.606 which is just a spontaneous pathway.
00:56:45.610 --> 00:56:48.522 So it’s well known in the old sort
00:56:48.522 --> 00:56:51.487 of Gina toxin literature that N
00:56:51.487 --> 00:56:54.172 3 adenine addicts are unstable
00:56:54.172 --> 00:56:55.930 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 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 an analog of Kohli Bakhtin. The differences are highlighted in green, and so the dye keten Kohli backed in on the top here is very unstable. You can’t work with this compound, it would be, you know Sufficient 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 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. What he’s doing right now is basically looking to see if this induces the same
type of mutational signature that one gets with the bacteria and that will be, you know, sort of the end. The end point. Hopefully you know it will be a positive result, but whatever it is that will be sort of the endpoint for this. And so I just acknowledge all the people that did the work. I'll just go through this quickly to save time. But this is my collaborator Jason. Many of you know him. 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
NOTE Confidence: 0.8687447
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

NOTE Confidence: 0.8513931

96
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, and that’s sort of the phase that we’re entering into with the project traffic we are after. The project traffic we are after.

1:00:45.550 --> 1:00:47.958 Yeah, there’s a lot that we can do, and that’s sort of the phase that we’re entering into with the project traffic we are after. The project traffic we are after.

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1:00:45.550 --> 1:00:47.958 Yeah, there’s a lot that we can do, and that’s sort of the phase that we’re entering into with the project traffic we are after. The project traffic we are after.
you thanks everyone.