My pleasure to present our next speaker, Dr. Ranjit Bindra, who is an Associate Professor of Therapeutic Radiology here at Yale School of Medicine.

Dr. Bindra is a graduate of Yale School of Medicine, so we are very proud of that. And he received his MD and PhD in this program. And he has also completed his residency in Radiation Oncology at Sloan-Kettering Cancer Center.

Since then he has come back home and has been an extremely successful and accomplished physician scientist with many discoveries that are now finding their way to clinic.

Today, he is going to talk to us about how he’s exploiting some metabolic vulnerabilities in gliomas that have a BMP1D mutation.

I give you Dr. Bindra.

Thank you very much.

Okay, great.

Okay, great.

Thanks a lot for having me today.

I want to tell you about a really interesting recent story from our group looking at DIPG mutation and its effect actually on any de-metabolism.

These are my disclosures which are not relevant today. We’ll start off just with one slide.

really on sort of our approach to novel therapeutics development here at the cancer center.
We'll then move on to the story of a DIBG-associated mutation in this gene called PPM1D and how it actually affects a NAD metabolism and leads to a clinically actionable target. Then if time permits, we'll cover a little bit about how we're trying to translate this directly into the clinic like we've done before.

We are very interested in bench to bedside discoveries and studies in our laboratory. And a lot of it starts with looking at the landscape of tumor-associated mutations like the ones that are shown here. We like to look at those mutations and figure out rapid and effective ways to model them, so often we'll use Cripsr Cast, but often we'll just use things like simple open reading frame expression just so we can get isogenic modeling of each one of these mutations. We then move those model cell lines into synthetic lethal screens.

One of the unique things that we’re very, very interested in is trying to find the sort of Achilles Heels. So trying to find driver mutations that may induce defects that we can then exploit for therapeutic gain.

We then move towards more patient-derived,
more relevant cell line models to validate the effects from our screens and our isogenic cell lines. And of course we have to move this into flank and in vivo type modeling before we can actually move this into clinic. And finally, as I’ve mentioned earlier, we’re very interested in trying to drive our discoveries as quickly as possible, namely into Phase 1 and Phase 2 trials. Brain tumors being the bulk of our work, often we have drug delivery problems, and so often we’ll look to folks like the Saltzman Laboratory to explore alternate methods to deliver some of these drugs into the brain. And we’ve been working for quite some time with Mark’s group on nano-particle versions of some of the drugs that we’re studying. So with that sort of backdrop, let me give you a little overview of this story. First we need to start with DIPG. This is a disease that I am actually relatively obsessed with having seen my first patient at Sloan-Kettering and watching that 3-year-old patient die was really touching for me. For the clinicians in the room, you know these films quite well. For the non-clinicians, this is an Axial T2 MRI, and then this is just to orient you.
for the non-clinicians.

This is very, very devastating tumor here in the brainstem, which largely can be regarded as the Grand Central Station for the human body.

And these tumors literally will take a child’s life within about 2 years.

Okay?

And a picture is worth a thousand words, and so I often like to show the pictures of patients that we’ve lost in our clinic to this disease to understand that we need to do something better. This child lasted about 2 years.

On average, a patient with DIPG in 1990 would live about 9 months.

So in the last 20 years, we’re still at about 9 months. It’s actually quite depressing.

And one of the things to note here is that biopsies in this disease are quite rare.

This is a very difficult area to get tissue, and so much of the treatments were based on diagnostic MRI images, then with the assumption that these are just baby versions of adult gliomas.

Once we began biopsying these tumors, folks like Chris Coley in Neurosurgery Pediatrics here, who did a lot of these biopsies when he was a fellow up in Boston, we suddenly realized that these were not adult tumors.

These were very, very unique.
The spectrum mutations were quite different.
Some of you may recognize one of these mutations. This is a H3K27M mutation that’s found in about 80 percent of DIPGs.
This gene mutation profoundly affects chromatin structure and leads to enormous range of gene expression and changes in the cell. But a subset of these, these tumors also have the mutations in a phosphatase called PPM1D.
So what’s the role of PPM1D in DIPG?
We’ll get to that in just a moment.
What I’ll tell you is, over the last 10 years or so, there’s no known role in epigenetic regulation for PPM1D.
So just zooming in on this mutation.
This is a phosphatase as I mentioned.
And in 2014, so five years ago, Hyan and colleagues at Duke showed that these mutations cluster in the C-terminal domain. They’re heterozygous, and they’re activating. So they lead to a hyper stable version of this phosphatase.
And interestingly, even though this gene was implicated in DIPG 5 years ago, we’ve known about this gene for actually about 20 years.
Actually back in ’97.
This gene was also known as Wild-type p53-induced phosphatase 1.
So these are the same gene.
And these genes are actually implicated in things like breast cancer as well as ovarian cancer and neuroblast.
The difference is that the gene is actually amplified in these cases versus a hyper stable activation via the heterozygous mutation here.
So what do these mutations do? PPM1D is actually involved in dephosphorylating the SQT motif modifications induced by ATM and ATR.
And these are the types of proteins that are targeted by PPM1D shown here.
One of the most commonly or well-established targets is H2AX, so hyperactive PPM1D actually leads to an accelerated dephosphorylation of H2AX.
So it’s thought to in principle disrupt the DNA repair and DNA response.
So from our perspective, for our laboratory, there’s sort of a fork in the road.
How do we target these mutations, right? So on one end,
we could just block aberrant phosphatase activity, right?
And those that know our lab and IDH1 story, we don’t like doing that, okay?
And there are drugs that have been developed.
Actually for the last 10 or 12 years,
there’s about 3 or 4 drugs that have been developed that simply block the phosphatase activity.
Most of them are not drug-like, none are in clinical trials, and overall they haven’t been that effective as an anti-tumor strategy for tumors that have these types of mutations. So we’re, again, very interested in exploiting Achilles Heels, or tumor-associated defects, hopefully by DNA repair given the role of this mutation in DNA repair. 

So with that, entered our first graduate student in the laboratory several years ago, Nate Fons. And Nate set out to model the PPM1D mutation, and to simply ask a question whether we could do a drug screen with an isogenic cell lines. It actually took him about a year and half to make this model, and this is shown here. This is a truncated activated form. We targeted that C-terminal domain where the DIPG mutations are found. And you can see this hyper activated, or of high levels of expression by western blot. And he did all the things a good grad student should, which is looked at protein stability and confirmed indeed that this is a hyper stable form of the protein. And he did functional these to show that this mutation was active in the sense that post-IR could get an accelerated de-phosphorylation of H2AX,
and this was dependent upon PPM1D activity because treatment with a PPM1D inhibitor abolished that effect. And this is just a FOSI example shown here. Then Nate, after about a year and a half, went on to do a screen, and we used the platform that we developed to find the IDH induced PARP sensitivity that some of you heard me talk about before. This is a 96 well plate medium throughput viability screen that we developed. And we were super excited because our idea was that we were going to essentially get, IDH impairment sensitivity, PPM1D hyperactive dis-regulation of DNA repair, that we would get another hit in that class. So Nate looked at about 100 DNA repair inhibitors and DNA damaging agents. And to our surprise, we found nothing, which that was always really stressful when it’s your first graduate student, and that’s their screen after 2 years, right? So it’s a tough thesis meeting. However, it turns out that we had one extra row in the 96 well plate. I just love telling this story because it’s sort of the story of how academia often operates. We had one extra row, and I was actually doing the plating.
back in the day and the folks in my lab just said remind that I was in the laboratory, and I actually had plated, we had one extra row and we put in some NAMPT, a NAMPT inhibitor row based on a paper by Dan Cahill up in Boston. He had shown that IDH mutations, again our laboratory is very interested in those, those mutations as well. He had shown that IDH mutations confer sensitivity to the NAMPT inhibitors via this NAD depletion phenotype. And this is the drug we added to this, this set of plates. Oddly enough, that was the only hit in our screen, which was very surprising to us. So what is NAD, and what are NAMPT inhibitors? This is a pathway. Again, when we worked on the IDH stuff, we actually had to relearn the citric acid cycle, and here we had to learn about NAD during the course of this work. And this is the NAD sort of cycle, and there’s multiple different ways to generate NAD which is sort of the central currency of life in a metabolizing cell. And so the first thing we did was actually just cold called a guy named Charlie Brenner. He’s out at Iowa, and he discovered a very, very critical pathway in the NAD biosynthetic pathway. And we called and we said we’ve got this very odd
0:10:09.16 –> 0:10:12.08 PPM1D induced NAMPT inhibitor sensitivity,
0:10:12.08 –> 0:10:13.05 can you help us out?
0:10:13.05 –> 0:10:14.43 And just to orient folks,
0:10:14.43 –> 0:10:17.78 NAMPT is a critical player in the NAMPT salvage pathway
0:10:17.78 –> 0:10:21.93 that essentially regenerates NAD and it’s
0:10:21.93 –> 0:10:24.62 blocked by these drugs called NAMPT inhibitors.
0:10:24.62 –> 0:10:26.05 So just sort of Cliff notes, and again,
0:10:26.05 –> 0:10:27.82 aging myself by using Cliff notes
0:10:27.82 –> 0:10:29.66 because I know about 90 percent of the audience
0:10:29.66 –> 0:10:31 does not know what these are.
0:10:31 –> 0:10:32.62 Nut these were very, very useful
0:10:32.62 –> 0:10:34.22 before the days of Google.
0:10:34.22 –> 0:10:36.61 And so NAMPT inhibitors are interesting drugs.
0:10:36.61 –> 0:10:38.49 There’s actually a diverse range of drugs out there.
0:10:38.49 –> 0:10:39.83 They’re highly potent.
0:10:39.83 –> 0:10:42.5 They’ve actually been tested in Phase 1 and 2 trials.
0:10:42.5 –> 0:10:44.42 There’s still a few
0:10:44.42 –> 0:10:45.66 drugs that are being tested.
0:10:45.66 –> 0:10:47.08 Most have actually been shelved
0:10:47.08 –> 0:10:48.89 because there really is no biomarker.
0:10:48.89 –> 0:10:50.21 There’s actually a lot of toxicity
0:10:50.21 –> 0:10:52.6 in the face of limited efficacy.
0:10:52.6 –> 0:10:54.05 So with that sort of backdrop,
0:10:54.05 –> 0:10:56.9 Nate went on to probe this interaction further.
0:10:56.9 –> 0:11:00.36 He first ruled out any clonal artifact from CRISPR,
0:11:00.36 –> 0:11:02.1 and he showed a multiple CRSPR clones that
0:11:02.1 –> 0:11:05.35 we had very nice NAMPT sensitivity in the PPM1D mutants.
0:11:05.35 –> 0:11:06.93 He then showed it was a class specific,
0:11:06.93 –> 0:11:08.65 not just a drug effect.
He showed that with multiple, structurally unique NAMPT inhibitors that we could still get mutant PPM1D induced differential sensitivity. And then as I mentioned earlier, we had the activating truncating mutations as well as the amplifications. He went on to show that overexpression of both full-length or truncated PPM1D could also recapitulate the NAMPT sensitivity. Uh, in contrast, a catalytically inactive version of PPM1D was unable to confer NAMPT inhibitor sensitivity. So we then sent ourselves to Charlie Brenner’s developed, high resolution NAD metabolic profiling platform. And he sent us back some intriguing data in that really all the NAD precursors were suppressed. And at base line you can see here Wild site versus the PPM1D mute. You can see base line, uh, depressed levels. When you treat with a NAMPT inhibitor, then you get critically low levels of NAD which we believe is contributing to the loss of viability in those cells. So then zooming in on this. We worked with Charlie, uh, to sort of probe the mechanistic basis for this phenomenon. Charlie suggested that we start repleting or rescuing, with various precursors. Adding NAM, adding NR, and adding NA to test the integrity of each of these pathways.
Okay?

So, these are synergy or antagonism plots that I’m showing you right here.

So, this is the drug NAMPT inhibitor, and then this is the NAD precursor that we’re adding. Red indicates an antagonistic effect, essentially showing that that pathway is intact.

Adding NAM you can see then bypasses the effect of the NAMPT inhibitor, so that pathway essentially was intact. Adding NR, his favorite NAD precursor also led to antagonism.

But the one intriguing result was shown here on the left. When you add NA, we’re unable to antagonize, suggesting the defect in this pathway to converge with NAMN which is mediated by this protein called NAPRT.

In parallel, Nate then did a siRNA screen knocking down each one of these drugs to see which one would phenocopy the PPM1D mutation causing NAMPT inhibitor sensitivity.

And he found one gene target of interest. And indeed that was NAPRT.

We then rushed back to our cell lines and asked the question, what is the status of NAPRT expression in these cell lines?
Maybe there’s a problem with it. And to our surprise, in all of the lines that had engineered a PPM1D mutation, they had lost NAPRT expression under these conditions. We then went ahead and said well is NAPRT loss accounting for the NAMPT sensitivity? So he over expressed NAPRT in the PPM1D mutant cells, and that’s shown here in the blue bar, so they completely rescue the effect. So this is really being driven by loss of NAPRT. We then moved again in our process flow to patient-derived models which obviously are more relevant to the human situation. And we got some patient-derived 3D DIPG cultures from Michelle Monje out at Stanford. And you can see here again in the mutant PPM1D cultures shown here that we had loss of NAPRT. So we could recapitulate, we could see this also in patient-derived models, and that led to profound sensitivity to a NAMPT inhibitor. And that’s shown here, and again, just by eying these 3D cultures, it’s quite striking. Working with Ranjini our fearless lab manager in the lab, we developed a PPM1D mutant flank xenograph model. And then we also showed
that this effect could be recapitulated in vivo in this flank model shown here.

Now narrowing in on the mechanism.

So we ask, well the protein is down so what exactly is happening?

This is not thought to be an epigenetic modifier,

But could this be possible?

So here’s a Tacksman analysis of MRI transcript levels.

You can see here we have reduction of, uh, of NAPRT levels.

in our PPM1D mutant engineered and patient-derived lines.

We then went and did a series of ChIP Assays at pretty comprehensive panel looking at the promoter,

which I won’t show you today that suggested that there was some sort of repressive effect of the promoter.

And then more importantly,

we showed that there was elevated 5 methylcytosine directly at the NAPRT promoter.

And this is just a methyl-dip assay.

Again, just glossing over this because of time.

But this really suggested to us that the promoter’s actually being silenced by mutant PPM1D.

So we sought to probe this a little bit deeper,

I’ll show you just a little smattering of the data that, uh, we’ve gotten more recently.

Uh, so we brought in a bioinformatics group and did whole methylene profiling to understand whether this was focal or global.
Uh, we actually expanded our patient-derived line. There’s sets of lines. There’s actually only a handful of PPM1D mutant DIPG lines in the world, and we are able to get them. And then we sort of looked and asked the question of whether this was a specific, uh, NAPRT promoter specific, or a global methylation, uh, phenotype. Uh, so we brought in the folks from TGEN. We’ve been working with Mike Berens for quite some time, and asked them to join. And then we reached out to folks across the pond, namely Chris Jones and the Carcaboso Lab, who some of these PPM1D patient-derived models for some of our work. What we first found looking at 850K, you can see in this red for the beta values, that largely the PPM1D mutants had a focal, dense hyper methylation of the NAPRT promoter. And actually when you look at global methylation profiling, you can see that on average, again, yellow are the mutant lines. You can see this cluster of methylation targets, essentially a CPG island like methylene phenotype that we’re seeing in the PPM1D mutants. Again, we’re seeing this both in the patient-derived lines as well as in our engineered lines in this systems.
So just sort of our working model. This was just published about two weeks ago in Nature Communications. What we’re finding is that elevated PPM1D activation leads to silencing of NAPRT likely in the context of a CPG island like methylene phenotype, which in activates this press handler salvage pathway essentially silencing NAPRT leading to the depletion of NAD and a setup, essentially a metabolic vulnerability for treatment with NAMPT inhibitors. There’s a lot more work to be done here, and because of time, I won’t go into those questions, but this work is really just beginning for us. Bringing it now back to IDH1, so some of you know some of the adult midline supratentorial gliomas have IDH mutations. And there’s a really an intriguing leak, link between PPM1D and IDH1. I alluded to this earlier from the Dan Cahill work that actually prompted us to serendipitously sort of make this discovery. And what, what Dan and colleagues actually found was similarly in IDH mutants as well, they silence NAPRT leading to an NAD depletion. So we don’t understand why adult and pediatric tumors with these mutations are silencing this pathway, but there’s clearly a theme across all age groups for these tumors for NAD depletion. So in the last just 5 minutes or so,
I'll tell you about what we're doing to get this into the clinic. So as many of you know we are very interested in trying to drive some of the work that we do into patients as soon as possible. And this is work that I think many of you seen us present, and this is work from the Glazer Lab, Stephanie Halene’s lab, Morokinaw, and my laboratory, essentially mapping out this oncometabolite-induced brachinist that leads to NAPRT sensitivity. And so we've done this before, and we've been able to translate this work into multiple clinical trials shown here. And really a testament to the cancer center, namely folks like, uh, Pat Lorusso, Paul Eder, Asher Marks, Toma Tebaldi, and again Stephanie Halene to really drive this into our patients. So the questions for this were how we're going to get this into the clinic, recognizing some of these huge caveats that I’m going spend the last few minutes on. So first of all, there are a number of barriers to a systemic NAMPT inhibitor trial, uh, in DIPG that we’ll touch upon in a moment. We would love to consider combinations with both radiation and chemotherapy because we don’t think monotherapy for any of these, these aggressive gliomas is going to be sufficient. And I’ll tell you a little bit about some surprising
results about the blood brain barrier penetration of some of the drugs that are out there.

So just a few, uh, few points on the first question.

So, as I mentioned, multiple NAMPT inhibitor trials have been initiated and closed.

Most of them ended with lack of efficacy, and pretty significant doxylamine toxicity.

A lot of folks would say that the lack of efficacy was simply that these were solid tumor Phase 1 trials with no biomarkers.

They were not trying to find for any specific biomarker that could confer sensitivity.

And the liabilities in particular were hemologic and retinal toxicity.

which have really spooked a lot of folks that are, developing NAMPT inhibitors at the moment, and they’ve shelved them.

This is just one paper to show you an example of, of this finding.

So, in parallel to that, we’d love to explore the concept of combining this with other clinically relevant regimens for glioma, namely DIPG.

And it turns out as many of you know in the audience here, temozolomide is a mainstay of brain tumor treatment. And temozolomide itself actually has been shown to cause an NAD depletion by metabolic stress.

In parallel, what about things like radiation, another mainstay for DIPG and other gliomas?
And I do apologize for I rat out colleagues I know to quote a paper from 1978. I promise I’m going to get a more recent one. But it turns out that radiation actually depletes NAD levels as well. And so where am I going with this? We, we have now NAMPT inhibitors, possibly radiation temodar - those are, that’s like the, the stupe trial plus NAMPT inhibitor - so an opportunity for what I would call tri-modality synergy with NAMPT inhibitors. So we’re really excited about possibly incorporating these modalities into a future clinical trial. So the last little point, again I just want to give you a flavor for this because of time. There’s a lot more to it. What about CNS penetration? So, one thing we learn is that your drug is no, no better than how well it can get into the blood, past the blood brain barrier for glioma trials. Turns out that most NAMPT inhibitors are CNS impermeable. The ones that are permeable actually have that retina toxicity that I mentioned earlier. So this is a bit of a conundrum. And so one thing that we’re interested in looking at is Convection Enhanced Delivery. Some of you may this, may know of this approach where you directly inject a drug into the brainstem or into the brain to bypass the blood brain barrier.
Folks like Joe Piepmeier and colleagues, uh, have done pioneering work in this field. And believe it or not, this is actually now, now quite common. There's probably about 7 or 8 trials in kids and adults testing CED of novel agents. Uh, we would argue that this is a great idea, but we know within a few hours those drugs you inject, they wash right away. Um, and so if the way to encapsulate those drugs in some sort of particle, i.e. nano-particle, we could then find a way to prolong, uh, the delivery and exposure in the tumor. So who could we go to for that? Well, of course we could go right across the street to Mark Saltzman. And Mark and Jianbing Zhou and folks have, have really done pioneering work in developing brain penetrating PEG and related nano-particles and have shown in some really seminal papers including this one in PNAS, that you could use them to treat glioblastoma. So we've been working with Mark for quite some time. So some of you know over the last couple years we've had a very, a long fruitful collaboration. We've actually shown by proof of concept that we could take DNA repair inhibitors, like ATR inhibitors, uh, and encapsulate them in nano-particles and use them to treat, gliomas. And this is just one of our papers that came out recently.
So that’s actually exactly what we’re doing now for NAMPT inhibitors. And this is actually a YCC co-pilot grant looking at whether we can capsulate NAMPT inhibitors in nano-particles. And this is work from Yazhe Wang and Jason Breckta, radunct resident in my laboratory showing that yes, we can and that these particles effectively can release drug and actually deplete NAD in this setting. So just to wrap up here in the last 2 minutes. So, we really are firm believers that metabolic vulnerabilities can be exploited in both adult and pediatric gliomas. We’ve shown this for IDH in the adults, and now we’re showing for PPM1D in the kids. We believe that just like IDH, we’re trying to translate this into the clinic. We’re really falling up as fast as we can to understand why PPM1D mutations are inducing NAPRT silencing. And, we do believe that there’s an opportunity here to take existing treatments like radiation and temodar and bring in NAMPT inhibitors into the fray. And we’re very actively exploring whether CED and nano-particles may address some of the issues that I’ve talked about earlier. So with that I’ll just wrap up.

I’ll thank all the folks that did the work in the laboratory, and all of them are shown here.
at our recent retreat.
Nate has moved on.
He’s now our first, first grad student,
and now a post-doc at the NCI.
And of course I’d like to thank the folks that
fund this work as well.
And we have time for a few questions.