## WEBVTT

1 00:00:00.180 --> 00:00:02.030 <v ->My pleasure to present our next speaker,</v>

2 00:00:02.030 --> 00:00:05.340 Dr. Ranjit Bindra, who is an Associate Professor

3 00:00:05.340 --> 00:00:09.910 of The<br/>rapeutic Radiology here at Yale School of Medicine.

4 00:00:09.910 --> 00:00:12.310 Dr. Bindra is a graduate of Yale School of Medicine,

 $5\ 00:00:12.310 \longrightarrow 00:00:14.340$  so we are very proud of that.

 $6~00{:}00{:}14.340$  -->  $00{:}00{:}18.720$  And he received his MD and PhD in this program.

7 00:00:18.720  $\rightarrow$  00:00:21.100 And he has also completed his residency

8 00:00:21.100 --> 00:00:24.690 in Radiation Oncology at Sloan-Kettering Cancer Center.

 $9~00{:}00{:}24.690 \dashrightarrow 00{:}00{:}27.700$  Since then he has come back home and has been

 $10\,00{:}00{:}27.700 \dashrightarrow > 00{:}00{:}30.990$  an extremely successful and accomplished physician scientist

11 00:00:30.990 --> 00:00:33.380 with many discoveries that are now finding their way

 $12\ 00:00:33.380 \longrightarrow 00:00:34.880$  to clinic.

13 00:00:34.880 --> 00:00:37.150 Today, he is going to talk to us

14 00:00:37.150 --> 00:00:41.670 about how he's exploiting some metabolic vulnerabilities

 $15\ 00:00:41.670 \longrightarrow 00:00:46.260$  in glimoas that have a BMP1D mutation.

16 00:00:46.260 --> 00:00:47.480 I give you Dr. Bindra.

17 00:00:47.480  $\rightarrow 00:00:48.591$  Thank you very much.

18 00:00:48.591 --> 00:00:50.841 (applause)

19 00:01:04.030 --> 00:01:04.863 <v ->Okay, great.</v>

 $20\ 00:01:04.863 \longrightarrow 00:01:06.950$  Thanks a lot for having me today.

21 00:01:06.950 --> 00:01:09.230 I want to tell you about a really interesting

22 00:01:09.230 --> 00:01:12.710 recent story from our group looking at DIPG mutation

 $23\ 00:01:12.710 \longrightarrow 00:01:15.700$  and its effect actually on any de-metabolism.

24 00:01:15.700 --> 00:01:18.310 These are my disclosures which are not relevant today.

 $25\ 00:01:18.310 \longrightarrow 00:01:20.050$  We'll start off just with one slide

 $26\ 00:01:20.050 \longrightarrow 00:01:21.810$  really on sort of our approach to

27 00:01:21.810 --> 00:01:25.280 novel the<br/>rapeutics development here at the cancer center.

 $28\ 00:01:25.280 \longrightarrow 00:01:26.700$  We'll then move on to the story

29 00:01:26.700 --> 00:01:29.667 of a DIBG-associated mutation in this gene called PPM1D

 $30\ 00{:}01{:}29.667 \dashrightarrow 00{:}01{:}32.570$  and how it actually affects a NAD metabolism

31 00:01:32.570  $\rightarrow 00:01:35.500$  and leads to a clinically actionable target.

 $32\ 00:01:35.500 \rightarrow 00:01:37.460$  Then if time permits, we'll cover a little bit

33 $00{:}01{:}37.460 \dashrightarrow 00{:}01{:}39.260$  about how we're trying to translate this

 $34\ 00:01:39.260 \longrightarrow 00:01:42.660$  directly into the clinic like we've done before.

35 00:01:42.660 --> 00:01:43.570 So, just getting started.

 $36\ 00:01:43.570 \longrightarrow 00:01:46.061$  We are very interested in bench to be dside

37 00:01:46.061 --> 00:01:48.850 discoveries and studies in our laboratory.

38 00:01:48.850 --> 00:01:51.380 And a lot of it starts with looking at the land-scape

 $39\ 00:01:51.380 \longrightarrow 00:01:52.910$  of tumor-associated mutations

 $40\ 00:01:52.910 \longrightarrow 00:01:55.480$  like the ones that are shown here.

41 00:01:55.480 --> 00:01:56.800 We like to look at those mutations

 $42\ 00:01:56.800$  --> 00:01:59.690 and figure out rapid and effective ways to model them,

43 00:01:59.690 --> 00:02:01.970 so often we'll use Cripsr Cast,

44 00:02:01.970 --> 00:02:03.280 but often we'll just use things like

 $45\ 00:02:03.280 \longrightarrow 00:02:05.060$  simple open reading frame expression

46 00:02:05.060 --> 00:02:07.430 just so we can get isogenic modeling of each one

 $47\ 00:02:07.430 \longrightarrow 00:02:08.940$  of these mutations.

 $48\ 00:02:08.940 \longrightarrow 00:02:10.800$  We then move those model cell lines

49 00:02:10.800 --> 00:02:12.300 into synthetic lethal screens.

 $50\ 00{:}02{:}12.300 \dashrightarrow 00{:}02{:}15.500$  Often we'll combine them with DNA damaging agents as well.

51 00:02:15.500 --> 00:02:18.090 One of the unique things that we're very, very interested in

 $52\ 00:02:18.090 \rightarrow 00:02:20.860$  is trying to find the sort of Achilles Heels.

53 00:02:20.860 --> 00:02:23.520 So trying to find driver mutations that may induce defects

54 00:02:23.520 --> 00:02:26.322 that we can then exploit for the rapeutic gain.

 $55\ 00:02:26.322 \longrightarrow 00:02:29.140$  We then move towards more patient-derived,

56 00:02:29.140 --> 00:02:31.556 more relevant cell line models to validate the effects

57  $00:02:31.556 \rightarrow 00:02:34.250$  from our screens and our isogenic cell lines.

 $58\ 00:02:34.250 \longrightarrow 00:02:36.050$  And of course we have to move this

59 00:02:36.050 --> 00:02:38.480 into flank and in vivo type modeling

 $60\ 00:02:38.480 \longrightarrow 00:02:40.300$  before we can actually move this into clinic.

61 00:02:40.300 --> 00:02:41.690 And finally, as I've mentioned earlier,

 $62\ 00{:}02{:}41.690$  -->  $00{:}02{:}44.030$  we're very interested in trying to drive our discoveries

 $63\ 00:02:44.030 \longrightarrow 00:02:45.480$  as quickly as possible,

64 00:02:45.480 --> 00:02:47.890 namely into Phase 1 and Phase 2 trials.

65 00:02:47.890 --> 00:02:50.220 Brain tumors being the bulk of our work,

66 00:02:50.220 --> 00:02:51.880 often we have drug delivery problems,

 $67\ 00:02:51.880 \longrightarrow 00:02:54.430$  and so often we'll look to folks

 $68\ 00{:}02{:}54{.}430$ --> $00{:}02{:}57{.}580$  like the Saltzman Laboratory to explore alternate methods

 $69\ 00:02:57.580$  --> 00:02:59.680 to deliver some of these drugs into the brain.

 $70\ 00:02:59.680 \longrightarrow 00:03:02.184$  And we've been working for quite some time

71 00:03:02.184  $\rightarrow 00:03:03.780$  with Mark's group on nano-particle versions

 $72\ 00:03:03.780 \longrightarrow 00:03:05.960$  of some of the drugs that we're studying.

73 00:03:05.960 -> 00:03:07.070 So with that sort of backdrop,

 $74\ 00:03:07.070 \longrightarrow 00:03:10.090$  let me give you a little overview of this story.

 $75\ 00:03:10.090 \longrightarrow 00:03:12.070$  First we need to start with DIPG.

76 00:03:12.070 --> 00:03:12.903 This is a disease

 $77\ 00:03:12.903 \longrightarrow 00:03:14.741$  that I am actually relatively obsessed with

 $78\ 00:03:14.741 \longrightarrow 00:03:17.500$  having seen my first patient at Sloan-Kettering

79 $00:03:17.500 \dashrightarrow 00:03:19.860$  and watching that 3-year-old patient die

 $80\ 00:03:19.860 \longrightarrow 00:03:21.280$  was really touching for me.

81 00:03:21.280 -> 00:03:22.842 For the clinicians in the room,

 $82\ 00:03:22.842 \longrightarrow 00:03:25.390$  you know these films quite well.

 $83\ 00:03:25.390 \longrightarrow 00:03:26.540$  For the non-clinicians,

84 00:03:26.540 --> 00:03:28.530 this is an Axial T2 MRI,

 $85\ 00:03:28.530 \longrightarrow 00:03:29.770$  and then this is just to orient you

86 00:03:29.770 --> 00:03:31.530 for the non-clinicians.

 $87\ 00:03:31.530 \longrightarrow 00:03:34.170$  This is very, very devastating tumor

88 00:03:34.170 --> 00:03:35.010 here in the brain<br/>stem,

89 $00{:}03{:}35{.}010$ --> $00{:}03{:}38{.}290$  which largely can be regarded as the Grand Central Station

 $90\ 00:03:38.290 \longrightarrow 00:03:39.720$  for the human body.

91 00:03:39.720 --> 00:03:43.420 And these tumors literally will take a child's life

 $92\ 00:03:43.420 \longrightarrow 00:03:45.170$  within about 2 years.

93 00:03:45.170 --> 00:03:46.003 Okay?

94 00:03:46.003  $\rightarrow$  00:03:47.500 And a picture is worth a thousand words,

95 00:03:47.500 --> 00:03:49.450 and so I often like to show the pictures of patients

96 00:03:49.450  $\rightarrow 00:03:51.590$  that we've lost in our clinic to this disease

97 00:03:51.590 --> 00:03:53.800 to understand that we need to do something better.

98 00:03:53.800 --> 00:03:55.880 This child lasted about 2 years.

99 $00{:}03{:}55{.}880 \dashrightarrow 00{:}03{:}59{.}960$  On average, a patient with DIPG in 1990 would live

 $100 \ 00:03:59.960 \longrightarrow 00:04:01.230$  about 9 months.

 $101 \ 00:04:01.230 \longrightarrow 00:04:02.570$  How are we doing?

 $102\ 00{:}04{:}02.570$  -->  $00{:}04{:}05.740$  So in the last 20 years, we're still at about 9 months.

 $103\ 00:04:05.740 \longrightarrow 00:04:07.600$  It's actually quite depressing.

104 00:04:07.600 --> 00:04:09.730 And one of the things to note here is that biopsies

 $105 \ 00:04:09.730 \longrightarrow 00:04:10.890$  in this disease are quite rare.

106 00:04:10.890 --> 00:04:13.890 This is a very difficult area to get tissue,

 $107 \ 00:04:13.890 \longrightarrow 00:04:15.750$  and so much of the treatments were based

108 00:04:15.750 --> 00:04:19.527 on diagnostic MRI images, then with the assumption

109 00:04:19.527 --> 00:04:24.140 that these are just baby versions of adult gliomas.

110 $00:04:24.140 \dashrightarrow 00:04:26.080$  Once we began biopsying these tumors,

111 00:04:26.080 --> 00:04:28.890 folks like Chris Coley in Neurosurgery Pediatrics here,

112 00:04:28.890 --> 00:04:30.630 who did a lot of these biopsies when he was a fellow

 $113\ 00:04:30.630 \longrightarrow 00:04:32.840$  up in Boston, we suddenly realized

114 00:04:32.840 --> 00:04:34.870 that these were not adult tumors.

 $115\ 00:04:34.870 \longrightarrow 00:04:35.980$  These were very, very unique.

116  $00:04:35.980 \rightarrow 00:04:38.150$  The spectrum mutations were quite different.

117 00:04:38.150 --> 00:04:40.420 Some of you may recognize one of these mutations.

118 $00:04:40.420 \dashrightarrow 00:04:43.800$  This is a H3K27M mutation that's found in

 $119\ 00:04:43.800 \longrightarrow 00:04:46.130$  about 80 percent of DIPGs.

120 00:04:46.130 --> 00:04:47.970 This gene mutation

121 00:04:47.970 --> 00:04:49.580 profoundly affects chromatin structure

 $122\ 00:04:49.580 \longrightarrow 00:04:52.020$  and leads to enormous range of gene expression

 $123\ 00:04:52.020 \longrightarrow 00:04:53.530$  and changes in the cell.

124 00:04:53.530 --> 00:04:56.830 But a subset of these, these tumors also have the mutations

 $125\ 00:04:56.830 \longrightarrow 00:04:58.503$  in a phosphatase called PPM1D.

 $126\ 00:04:59.520 \longrightarrow 00:05:01.990$  So what's the role of PPM1D in DIPG?

 $127\ 00:05:01.990 \longrightarrow 00:05:03.600$  We'll get to that in just a moment.

128 00:05:03.600 --> 00:05:06.590 What I'll tell you is, over the last 10 years or so,

129 00:05:06.590 --> 00:05:10.940 there's no known role in epigenetic regulation for PPM1D.

130 00:05:10.940 --> 00:05:12.900 So just zooming in on this mutation.

131  $00:05:12.900 \rightarrow 00:05:15.300$  This is a phosphatase as I mentioned.

 $132\ 00:05:15.300 \longrightarrow 00:05:18.440$  And in 2014, so five years ago,

133 00:05:18.440 --> 00:05:21.000 Hyan and colleagues at Duke showed that

134 00:05:21.000 --> 00:05:23.650 these mutations cluster in the C-terminal domain.

 $135\ 00:05:23.650 \longrightarrow 00:05:25.450$  They're heterozygous, and they're activating.

136 $00{:}05{:}25{.}450 \dashrightarrow 00{:}05{:}28{.}810$  So they lead to a hyper stable version of this phosphatase.

 $137\ 00:05:28.810 \longrightarrow 00:05:30.950$  And interestingly, even though

138 $00:05:30.950 \dashrightarrow 00:05:33.360$  this gene was implicated in DIPG 5 years ago,

139 $00{:}05{:}33{.}360 \dashrightarrow 00{:}05{:}36{.}320$  we've known about this gene for actually about 20 years.

140  $00:05:36.320 \rightarrow 00:05:39.070$  Actually back in '97.

141 00:05:39.070 --> 00:05:40.461 This gene was also known as

 $142\ 00:05:40.461 \longrightarrow 00:05:44.260$  Wild-type p53-induced phosphatase 1.

 $143\ 00:05:44.260 \longrightarrow 00:05:45.950$  So these are the same gene.

144 00:05:45.950  $\rightarrow 00:05:48.100$  And these genes are actually implicated

 $145\ 00:05:48.100 \longrightarrow 00:05:49.670$  in things like breast cancer

146 $00:05:49.670 \dashrightarrow 00:05:51.840$  as well as ovarian cancer and neuroblast

147 00:05:51.840 --> 00:05:53.380 and medullo<br/>blastoma.

148 00:05:53.380 --> 00:05:55.430 The difference is that the gene is actually amplified

 $149\ 00:05:55.430 \longrightarrow 00:05:58.010$  in these cases versus a hyper stable activation

150 00:05:58.010 --> 00:06:00.984 via the heterozygous mutation here.

 $151\ 00:06:00.984 \longrightarrow 00:06:03.530$  So what do these mutations do?

152 00:06:03.530 --> 00:06:05.580 So PPM1D is actually involved

153 00:06:05.580 --> 00:06:09.881 in dephosphorylating the SQT motif modifications

 $154\ 00:06:09.881 \longrightarrow 00:06:12.260$  induced by ATM and ATR.

155 00:06:12.260 --> 00:06:15.070 And these are the types of proteinst that are targeted

 $156\ 00:06:15.070 \longrightarrow 00:06:16.490$  by PPM1D shown here.

 $157\ 00:06:16.490 \rightarrow 00:06:19.257$  One of the most commonly or well-established

158 00:06:19.257 --> 00:06:23.670 targets is H2AX, so hyperactive PPM1D actually leads

 $159\ 00:06:23.670 \longrightarrow 00:06:26.060$  to an accelerated dephosphorylation of H2AX.

160 00:06:26.060 --> 00:06:29.249 So it's thought to in principle disrupt the DNA repair

161 00:06:29.249 --> 00:06:30.888 and DNA response.

162 00:06:30.888 --> 00:06:33.580 So from our perspective, for our laboratory,

 $163\ 00:06:33.580 \longrightarrow 00:06:35.490$  there's sort of a fork in the road.

164 00:06:35.490 --> 00:06:37.950 How do we target these mutations, right?

165 00:06:37.950 --> 00:06:38.783 So on one end,

166 00:06:38.783 --> 00:06:40.617 we could just block aberrant phosphatase activity, right?

167 00:06:40.617 --> 00:06:42.970 And so those that know our lab and IDH1 story,

 $168\ 00:06:42.970 \longrightarrow 00:06:45.020$  we don't like doing that, okay?

 $169\ 00:06:45.020 \longrightarrow 00:06:47.100$  And there are drugs that have been developed.

 $170\ 00:06:47.100 \longrightarrow 00:06:48.820$  Actually for the last 10 or 12 years,

171 00:06:48.820 --> 00:06:51.000 there's about 3 or 4 drugs that have been developed

 $172\ 00:06:51.000 \longrightarrow 00:06:53.430$  that simply block the phosphatase activity.

 $173\ 00:06:53.430 \longrightarrow 00:06:55.290$  Most of them are not drug-like,

174 00:06:55.290 --> 00:06:56.570 none are in clinical trials,

 $175\ 00:06:56.570 \rightarrow 00:06:58.950$  and overall they haven't been that effective

 $176\ 00:06:58.950 \longrightarrow 00:07:00.920$  as an anti-tumor strategy for tumors

 $177\ 00:07:00.920 \longrightarrow 00:07:02.930$  that have these types of mutations.

178 00:07:02.930 --> 00:07:04.100 So we're, again, very interested

179 00:07:04.100 --> 00:07:06.000 in exploiting Achilles Heels,

180 00:07:06.000 --> 00:07:08.649 or tumor-associated defects,

181  $00:07:08.649 \rightarrow 00:07:11.950$  hopefully by DNA repair given the role of this

 $182\ 00:07:11.950 \longrightarrow 00:07:13.883$  mutation in DNA repair.

183 00:07:14.860 --> 00:07:17.070 So with that, entered our first graduate student

 $184\ 00:07:17.070$  --> 00:07:18.940 in the laboratory several years ago, Nate Fons.

185 00:07:18.940 --> 00:07:21.790 And Nate set out to model the PPM1D mutation,

 $186\ 00:07:21.790 \longrightarrow 00:07:22.840$  and to simply ask a question

 $187\ 00:07:22.840 \longrightarrow 00:07:24.020$  whether we could do a drug screen

 $188\ 00:07:24.020 \longrightarrow 00:07:26.230$  with an isogenic cell lines.

189 $00:07:26.230 \dashrightarrow 00:07:27.910$  So it actually took him about a year and half

190 $00:07:27.910 \dashrightarrow 00:07:30.510$  to make this model, and this is shown here.

191 $00:07:30.510 \dashrightarrow 00:07:32.470$  This is a truncated activated form.

192 $00{:}07{:}32{.}470 \dashrightarrow 00{:}07{:}35{.}080$  We targeted that C-terminal domain

193 $00{:}07{:}35{.}080 \dashrightarrow 00{:}07{:}37{.}030$  where the DIPG mutations are found.

194 00:07:37.030 --> 00:07:38.890 And you can see this hyper activated, or

 $195\ 00:07:38.890 \longrightarrow 00:07:40.910$  of high levels of expression by western blot.

196 $00{:}07{:}40{.}910$  -->  $00{:}07{:}42{.}850$  And he did all the things a good grad student should,

197 $00{:}07{:}42.850$  -->  $00{:}07{:}45.550$  which is looked at protein stability and confirmed indeed

198 $00{:}07{:}45{.}550 \dashrightarrow 00{:}07{:}48{.}510$  that this is a hyper stable form of the protein.

199<br/> 00:07:48.510 --> 00:07:51.200 And he did funcuatz<br/>ie these to show

200 00:07:51.200 --> 00:07:53.630 that this mutation was active in the sense that

201 00:07:53.630 --> 00:07:57.020 post-IR could get an accelerated dephosphorylation of H2AX,

 $202\ 00:07:57.020$ -->00:07:59.660 and this was dependent upon PPM1D activity

 $203\ 00:07:59.660 \longrightarrow 00:08:01.690$  because treatment with a PPM1D inhibitor

204 00:08:01.690 --> 00:08:03.050 abolished that effect.

 $205\ 00:08:03.050$  --> 00:08:05.800 And this is just a FOSI example shown here.

206 00:08:05.800 --> 00:08:07.500 Then Nate, after about a year and a half,

 $207\ 00:08:07.500 \longrightarrow 00:08:10.300$  or 2 years or so, went on to do a screen,

 $208\ 00:08:10.300 \longrightarrow 00:08:12.140$  and we used the platform that we developed

209 00:08:12.140 --> 00:08:14.610 to find the IDH induced PARP sensitivity

 $210\ 00:08:14.610 \longrightarrow 00:08:16.800$  that some of you heard me talk about before.

211 00:08:16.800 --> 00:08:19.449 This is a 96 well plate medium throughput

 $212\ 00:08:19.449 \longrightarrow 00:08:21.760$  viability screen that we developed.

213 00:08:21.760 --> 00:08:23.040 And we were super excited

214 00:08:23.040 --> 00:08:26.265 because our idea was that we were going to essentially get,

215 00:08:26.265 --> 00:08:28.990 IDH impairment sensitivity,

216 00:08:28.990 --> 00:08:31.640 PPM1D hyperactive dis-regulation of DNA repair,

 $217\ 00:08:31.640$  --> 00:08:34.380 that we would get another hit in that class.

218 00:08:34.380 --> 00:08:36.377 So Nate looked at about 100 DNA repair inhibitors

219 00:08:36.377 --> 00:08:38.100 and DNA damaging agents.

220 00:08:38.100 --> 00:08:40.728 And to our surprise, we found nothing,

 $221\ 00:08:40.728 \longrightarrow 00:08:42.260$  which that was always really stressful

222 00:08:42.260 --> 00:08:43.420 when it's your first graduate student,

 $223\ 00:08:43.420 \longrightarrow 00:08:45.200$  and that's their screen after 2 years, right?

 $224\ 00:08:45.200 \longrightarrow 00:08:46.690$  So it's a tough thesis meeting.

225 00:08:46.690 --> 00:08:51.690 However, it turns out that we had one extra row

226 00:08:52.300 --> 00:08:53.460 in the 96 well plate.

227 00:08:53.460 --> 00:08:54.550 I just love telling this story

228 00:08:54.550 --> 00:08:57.530 because it's sort of the story of how academia often

 $229 \ 00:08:57.530 \longrightarrow 00:08:59.010$  operates.

230 00:08:59.010 --> 00:09:02.190 We had one extra row, and I was actually doing the plating

231 00:09:02.190 --> 00:09:03.960 back in the day and the folks in my lab just said

 $232\ 00:09:03.960 \longrightarrow 00:09:06.260$  remind that I was in the laboratory, and

233 00:09:06.260 --> 00:09:07.960 I actually had plated, we had one extra row

234 00:09:07.960 --> 00:09:11.510 and we put in some NAMPT, a NAMPT inhibitor row

235 00:09:11.510 --> 00:09:13.940 based on a paper by Dan Cahill up in Boston.

 $236\ 00:09:13.940 \longrightarrow 00:09:15.500$  He had shown that IDH mutations,

237 00:09:15.500 --> 00:09:17.570 again our laboratory is very interested in those,

238 00:09:17.570 --> 00:09:19.290 those mutations as well.

239 00:09:19.290 --> 00:09:21.750 He had shown that IDH mutations confer sensitivity

240 00:09:21.750 --> 00:09:23.634 to the NAMPT inhibitors

 $241\ 00:09:23.634 \longrightarrow 00:09:25.582$  via this NAD depletion phenotype.

242 00:09:25.582 --> 00:09:29.460 And this is the drug we added to this, this set of plates.

243 00:09:29.460 --> 00:09:31.650 Oddly enough, that was the only hit in our screen,

 $244\ 00:09:31.650 \longrightarrow 00:09:33.363$  which was very surprising to us.

245 00:09:34.240 --> 00:09:36.880 So what is NAD, and what are NAMPT inhibitors?

 $246\ 00:09:36.880 \longrightarrow 00:09:38.170$  This is a pathway.

247 00:09:38.170 --> 00:09:39.790 Again, when we worked on the IDH stuff,

 $248\ 00:09:39.790 \longrightarrow 00:09:41.670$  we actually had to relearn the citric acid cycle,

 $249\ 00:09:41.670 \longrightarrow 00:09:44.380$  and here we had to learn about NAD

 $250\ 00:09:44.380 \longrightarrow 00:09:45.730$  during the course of this work.

251 00:09:45.730 --> 00:09:48.340 And this is the NAD sort of cycle,

 $252\ 00:09:48.340$  --> 00:09:50.530 and there's multiple different ways to generate NAD

253 00:09:50.530  $\rightarrow 00:09:52.900$  which is sort of the central currency of life

 $254\ 00:09:52.900 \longrightarrow 00:09:55.110$  in a metabolizing cell.

 $255\ 00{:}09{:}55{.}110$  -->  $00{:}09{:}57{.}607$  And so the first thing we did was actually just

256 00:09:57.607 --> 00:09:59.040 cold called a guy named Charlie Brenner.

257 00:09:59.040 --> 00:10:02.170 He's out at Iowa, and he discovered a very, very

258 00:10:02.170 --> 00:10:06.490 critical pathway in the NAD biosynthetic pathway.

 $259\ 00:10:06.490 \longrightarrow 00:10:07.650$  And we called and we said 260 00:10:07.650 --> 00:10:09.160 we've got this very odd 261 00:10:09.160 --> 00:10:12.080 PPM1D induced NAMPT inhibitor sensitivity,  $262\ 00:10:12.080 \longrightarrow 00:10:13.050$  can you help us out? 263 00:10:13.050 --> 00:10:14.430 And just to orient folks, 264 00:10:14.430 --> 00:10:17.780 NAMPT is a critical player in the NAMPT salvage pathway  $265\ 00:10:17.780 \longrightarrow 00:10:21.930$  that essentially regenerates NAD and it's 266 00:10:21.930 --> 00:10:24.620 blocked by these drugs called NAMPT inhibitors. 267 00:10:24.620 --> 00:10:26.050 So just sort of Cliff notes, and again, 268 00:10:26.050 --> 00:10:27.820 aging myself by using Cliff notes 269 00:10:27.820 --> 00:10:29.660 because I know about 90 percent of the audience  $270\ 00:10:29.660 \longrightarrow 00:10:31.000$  does not know what these are. 271 00:10:31.000 --> 00:10:32.620 Nut these were very, very useful  $272\ 00:10:32.620 \longrightarrow 00:10:34.220$  before the days of Google. 273 00:10:34.220 --> 00:10:36.610 And so NAMPT inhibitors are interesting drugs. 274 00:10:36.610 --> 00:10:38.490 There's actually a diverse range of drugs out there. 275 00:10:38.490 --> 00:10:39.830 They're highly potent.  $276\ 00:10:39.830 \longrightarrow 00:10:42.500$  They've actually been tested in Phase 1 and 2 trials. 277 00:10:42.500 --> 00:10:44.420 There's still a few  $278\ 00:10:44.420 \longrightarrow 00:10:45.660$  drugs that are being tested. 279 00:10:45.660 --> 00:10:47.080 Most have actually been shelved  $280\ 00:10:47.080 \longrightarrow 00:10:48.890$  because there really is no biomarker. 281 00:10:48.890 --> 00:10:50.210 There's actually a lot of toxicity  $282\ 00:10:50.210 \longrightarrow 00:10:52.600$  in the face of limited efficacy. 283 00:10:52.600 --> 00:10:54.050 So with that sort of backdrop,  $284\ 00:10:54.050 \longrightarrow 00:10:56.900$  Nate went on to probe this interaction further. 285 00:10:56.900 --> 00:11:00.360 He first ruled out any clonal artifact from CRISPR,

286 00:11:00.360 --> 00:11:02.100 and he showed a multiple CRSPR clones that 287 00:11:02.100 --> 00:11:05.350 we had very nice NAMPT sensitivity in the PPM1D mutants.

 $288\ 00:11:05.350 \longrightarrow 00:11:06.930$  He then showed it was a class specific,

 $289\ 00:11:06.930 \longrightarrow 00:11:08.650$  not just a drug effect.

290 00:11:08.650 --> 00:11:10.710 He showed that with multiple, structurally unique

291 00:11:10.710 --> 00:11:12.500 NAMPT inhibitors that we could still get

292 00:11:12.500 --> 00:11:16.200 mutant PPM1D induced differential sensitivity.

293 00:11:16.200 --> 00:11:17.110 And then as I mentioned earlier,

294 $00{:}11{:}17{.}110 \dashrightarrow 00{:}11{:}19{.}540$  we had the activating truncating mutations

 $295\ 00:11:19.540 \longrightarrow 00:11:21.250$  as well as the amplifications.

 $296\ 00:11:21.250 \longrightarrow 00:11:23.130$  He went on to show that over expression

297 00:11:23.130 --> 00:11:26.930 of both full-length or truncated PPM1D could also

298 00:11:26.930 --> 00:11:29.000 recapitulate the NAMPT sensitivity.

299 00:11:29.000 --> 00:11:32.540 Uh, in contrast, a catalycally inactive version of PPM1D

300 00:11:32.540 --> 00:11:35.335 was unable to confer NAMPT inhibitor sensitivity.

 $301\;00{:}11{:}35{.}335 {--}{>}00{:}11{:}38{.}830$  So we then sent ourselves to Charlie Brenner's developed,

302 00:11:38.830 --> 00:11:42.750 high resolution NAD metabolic profiling platform.

303 00:11:42.750 --> 00:11:45.230 And he sent us back some intriguing data

304 00:11:45.230 --> 00:11:48.900 in that really all the NAD precursors were suppressed.

 $305\ 00:11:48.900 \longrightarrow 00:11:50.710$  And at base line you can see here Wild site  $306\ 00:11:50.710 \longrightarrow 00:11:52.030$  versus the PPM1D mute.

307 00:11:52.030 --> 00:11:54.450 You can see base line, uh, depressed levels.

30800:11:54.450 --> 00:11:55.920 When you treat with a NAMPT inhibitor,

 $309\ 00:11:55.920 \longrightarrow 00:11:58.790$  then you get critically low levels of NAD

 $310\ 00:11:58.790 \longrightarrow 00:12:01.950$  which we believe is contributing to the loss

 $311\ 00:12:01.950 \longrightarrow 00:12:03.890$  of viability in those cells.

 $312\ 00:12:03.890 \longrightarrow 00:12:06.130$  So then zooming in on this.

313 00:12:06.130 --> 00:12:10.010 We worked with Charlie, uh, to sort of probe

 $314\ 00:12:10.010 \longrightarrow 00:12:12.410$  the mechanistic basis for this phenomenon.

315 00:12:12.410 --> 00:12:15.590 Charlie suggested that we start repleting or rescuing,

 $316\ 00:12:15.590 \longrightarrow 00:12:16.960$  with various precursors.

317 00:12:16.960 --> 00:12:20.780 Adding NAM, adding NR, and adding NA to test the integrity

 $318\ 00:12:20.780 \longrightarrow 00:12:22.200$  of each of these pathways.

319 00:12:22.200 --> 00:12:23.033 Okay?

 $320\ 00:12:23.033 \longrightarrow 00:12:25.560$  So, these are synergy or antagonism plots

321 00:12:25.560 --> 00:12:26.700 that I'm showing you right here.

322 00:12:26.700 --> 00:12:28.127 So, this is the drug NAMPT inhibitor,

323 00:12:28.127 --> 00:12:31.390 and then this is the NAD precursor that we're adding.

324 00:12:31.390 --> 00:12:34.370 Red indicates an antagonistic effect,

325 00:12:34.370 --> 00:12:36.510 essentially showing that that pathway is intact.

326 00:12:36.510 --> 00:12:37.343 Okay?

327 00:12:37.343 --> 00:12:39.820 So adding NAM you can see then by passes the effect

328 00:12:39.820 --> 00:12:40.653 of the NAMPT inhibitor,

 $329\ 00:12:40.653 \longrightarrow 00:12:42.330$  so that pathway essentially was intact.

330 00:12:42.330 --> 00:12:46.020 Adding NR, his favorite NAD precursor

 $331\ 00:12:46.020 \longrightarrow 00:12:47.720$  also led to antagonism.

 $332\ 00:12:47.720 \longrightarrow 00:12:50.070$  But the one intriguing result

333 00:12:50.070 --> 00:12:51.490 was shown here on the left.

 $334\ 00:12:51.490 \longrightarrow 00:12:52.510$  When you add NA,

 $335\ 00:12:52.510 \longrightarrow 00:12:53.670$  we're unable to antagonize,

336 00:12:53.670 --> 00:12:56.270 suggesting the defect in this pathway to converge

337 00:12:56.270 --> 00:12:59.913 with NAMN which is mediated by this protein called NAPRT.

338 00:13:01.060 --> 00:13:03.468 In parallel, Nate then did a siRNA screen

339 00:13:03.468 --> 00:13:05.600 knocking down each one of these drugs

 $340~00{:}13{:}05{.}600 \dashrightarrow 00{:}13{:}08{.}950$  to see which one would phenocopy the PPM1D mutation

341 00:13:08.950 --> 00:13:10.900 causing NAMPT inhibitor sensitivity.

 $342\ 00:13:10.900 \longrightarrow 00:13:14.070$  And he found one gene target of interest.

343 00:13:14.070 --> 00:13:15.590 And indeed that was NAPRT,

 $344\ 00:13:15.590 \longrightarrow 00:13:17.390$  and that's shown here in the orange.

345 00:13:18.490 --> 00:13:21.400 We then rushed back to our cell lines and asked the question

 $346\ 00:13:21.400 \longrightarrow 00:13:23.090$  well, what is the status of NAPRT expression

 $347\ 00:13:23.090 \longrightarrow 00:13:23.923$  in these cell lines?

348 00:13:23.923 --> 00:13:24.790 Maybe there's a problem with it.

349 00:13:24.790 --> 00:13:25.710 And to our surprise,

350 00:13:25.710 --> 00:13:28.860 in all of the lines that had engineered a PPM1D mutation,

351 00:13:28.860 --> 00:13:33.420 they had lost NAPRT expression under these conditions.

 $352\ 00:13:33.420 \longrightarrow 00:13:35.280$  We then went ahead and said

 $353\ 00{:}13{:}35{.}280\ {-->}\ 00{:}13{:}38{.}480$  well is NAPRT loss accounting for the NAMPT sensitivity?

35400:13:38.480 --> 00:13:41.910 So he over expressed NAPRT in the PPM1D mutant cells,

 $355\ 00:13:41.910 \longrightarrow 00:13:43.350$  and that's shown here in the blue bar,

 $356\ 00:13:43.350 \longrightarrow 00:13:44.900$  so they completely rescue the effect.

357 00:13:44.900 --> 00:13:47.693 So this is really being driven by loss of NAPRT.

 $358\ 00:13:48.672 \longrightarrow 00:13:49.505$  (throat clearing)

 $359\ 00:13:49.505 --> 00:13:51.588$  We then moved again in our process flow

360 00:13:51.588 --> 00:13:53.000 to patient-derived models which obviously are more relevant

361 00:13:53.000 --> 00:13:54.970 to the human situation.

362 00:13:54.970 --> 00:13:57.440 And we got some patient-derived

363 00:13:57.440 --> 00:14:00.910 3D DIPG cultures from Michelle Monje out at Stanford.

364 00:14:00.910 --> 00:14:04.450 And you can see here again in the mutant PPM1D

365 00:14:04.450 --> 00:14:06.580 cultures shown here that we had loss of NAPRT.

 $366\ 00:14:06.580 \longrightarrow 00:14:07.730$  So we could recapitulate,

367 00:14:07.730 --> 00:14:10.500 we could see this also in patient-derived models,

368 00:14:10.500 --> 00:14:13.890 and that led to profound sensitivity to a NAMPT inhibitor.

369 00:14:13.890 --> 00:14:15.290 And that's shown here, and again,

370 00:14:15.290 --> 00:14:18.403 just by eying these 3D cultures, it's quite striking.

371 00:14:19.270 --> 00:14:21.790 Working with Ranjini our fearless lab manager in the lab,

372 00:14:21.790 --> 00:14:25.560 we developed a PPM1D mutant flank zeno-graph model.

 $373\ 00:14:25.560 \longrightarrow 00:14:27.050$  And then we also showed

 $374\ 00:14:27.050 \longrightarrow 00:14:29.810$  that this effect could be recapitulated in vivo

 $375\ 00:14:29.810 \longrightarrow 00:14:32.566$  in this flank model shown here.

376 00:14:32.566 --> 00:14:34.950 Now narrowing in on the mechanism.

 $377\ 00:14:34.950 \longrightarrow 00:14:35.783$  So we ask,

378 00:14:35.783 --> 00:14:37.770 well the protein is down so what exactly is happening?

379 00:14:37.770 --> 00:14:40.030 This is not thought to be an epigenetic modifier,

380 00:14:40.030 --> 00:14:41.230 this mutation.

 $381\ 00:14:41.230 \longrightarrow 00:14:42.810$  But could this be possible?

382 00:14:42.810 --> 00:14:45.610 So here's a Tacksman analysis of MRI transcript levels.

383 00:14:45.610 --> 00:14:49.095 You can see here we have reduction of, uh, of NAPRT levels,

 $384\ 00{:}14{:}49.095$  -->  $00{:}14{:}53.320$  in our PPM1D mutant engineered and patient-derived lines.

 $385\ 00{:}14{:}53{.}320$  -->  $00{:}14{:}55{.}810$  We then went and did a series of ChIP Assays

386 00:14:55.810 --> 00:14:58.430 at pretty comprehensive panel looking at the promoter,

387 00:14:58.430 --> 00:15:00.170 which I won't show you today that suggested that

388 00:15:00.170 --> 00:15:02.720 there was some sort of repressive effect of the promoter.

 $389\ 00:15:02.720 \longrightarrow 00:15:03.910$  And then more importantly,

390 00:15:03.910 --> 00:15:06.280 we showed that there was elevated 5 methylcytosine

391 00:15:06.280  $\operatorname{-->}$  00:15:08.020 directly at the NAPRT promoter.

39200:15:08.020 $\operatorname{-->}$ 00:15:09.820 And this is just a methyl-dip assay.

 $393\ 00:15:09.820 \longrightarrow 00:15:12.320$  Again, just glossing over this because of time.

 $394\ 00:15:12.320 \longrightarrow 00:15:13.700$  But this really suggested to us that

395 00:15:13.700 --> 00:15:16.070 the promoter's actually being silenced

 $396\ 00:15:16.070 \longrightarrow 00:15:17.963$  by mutant PPM1D.

 $397\ 00:15:19.460 \longrightarrow 00:15:21.640$  So we sought to probe this a little bit deeper,

398 00:15:21.640 --> 00:15:23.690 and I'll show you just a little smattering of the,

399 00:15:23.690 --> 00:15:26.340 of the data that, uh, we've gotten more recently.

 $400\ 00:15:26.340 \longrightarrow 00:15:27.890$  Uh, so we brought in a bioinformatics group

401 00:15:27.890 --> 00:15:30.430 and did whole methylene profiling to understand

 $402\ 00:15:30.430 \longrightarrow 00:15:32.500$  whether this was focal or global.

 $403\ 00{:}15{:}32.500 \dashrightarrow 00{:}15{:}34.640$  Uh, we actually expanded our patient-derived line.

404 00:15:34.640 --> 00:15:35.473 There's sets of lines.

405 00:15:35.473 --> 00:15:39.130 There's actually only a handful of PPM1D mutant DIPG lines

 $406\ 00:15:39.130 \longrightarrow 00:15:40.970$  in the world, and we are able to get them.

407 00:15:40.970 --> 00:15:43.130 And then we sort of looked and asked the question

408 00:15:43.130 --> 00:15:46.850 of whether this was a specific, uh, NAPRT promoter specific,

409 00:15:46.850 --> 00:15:49.600 or a global methylation, uh, phenotype.

 $410\ 00:15:49.600 -> 00:15:50.880$  Uh, so we brought in the folks from TGEN.

411 00:15:50.880 --> 00:15:52.700 We've been working with Mike Berens for quite some time,

 $412\ 00:15:52.700 \longrightarrow 00:15:54.540$  and asked them to join.

413 00:15:54.540 --> 00:15:57.020 And then we reached out to folks across the pond,

414 00:15:57.020 --> 00:15:59.410 namely Chris Jones and the Carcaboso Lab,

415 00:15:59.410 --> 00:16:02.420 who some of these PPM1D patient-derived models

416 00:16:02.420 --> 00:16:04.170 for some of our work.

417 00:16:04.170 --> 00:16:07.750 What we first soun- what we first found looking at 850K,

418  $00:16:07.750 \rightarrow 00:16:10.150$  whole methylene in profiling is shown here.

 $419\ 00:16:10.150 \longrightarrow 00:16:12.550$  You can see in this red for the beta values,

420 00:16:12.550 --> 00:16:16.030 that largely the PPM1D mutants had a focal,

421 00:16:16.030 --> 00:16:18.790 dense hyper methylation of the NAPRT promoter.

 $422\ 00{:}16{:}18.790$  --> 00:16:21.070 And actually when you look at global methylation profiling,

 $423\ 00:16:21.070 \longrightarrow 00:16:23.000$  you can see that on average, again,

424 00:16:23.000 --> 00:16:24.430 yellow are the mutant lines.

 $425\ 00:16:24.430 \longrightarrow 00:16:28.025$  You can see this cluster of methylation targets,

426 00:16:28.025 --> 00:16:31.180 essentially a CPG island like methylene phenotype

 $427\ 00:16:31.180 \longrightarrow 00:16:33.160$  that we're seeing in the PPM1D mutants.

428 00:16:33.160 --> 00:16:35.640 Again, we're seeing this both in the patient-derived lines

 $429\ 00{:}16{:}35{.}640$  -->  $00{:}16{:}39{.}200$  as well as in our engineered lines in this systems.

 $430\ 00:16:39.200 \longrightarrow 00:16:40.740$  So just sort of our working model.

431 00:16:40.740 --> 00:16:42.390 This was just published about two weeks ago 432 00:16:42.390 --> 00:16:43.770 in Nature Communications.

433 00:16:43.770 --> 00:16:46.770 What we're finding is that elevated PPM1D activation

434 00:16:46.770 --> 00:16:49.700 leads to silencing of NAPRT likely in the context

435 00:16:49.700 --> 00:16:52.080 of a CPG island like methylene phenotype,

436 00:16:52.080 --> 00:16:55.270 which in activates this press handler salvage pathway

437 00:16:55.270 --> 00:16:58.161 essentially silencing NAPRT leading to the depletion of NAD

438 00:16:58.161 --> 00:17:01.590 and a setup, essentially a metabolic vulnerability

 $439\ 00:17:01.590 \longrightarrow 00:17:03.740$  for treatment with NAMPT inhibitors.

 $440\ 00:17:03.740 \longrightarrow 00:17:06.010$  There's a lot more work to be done here,

441 00:17:06.010 --> 00:17:07.890 and because of time, I won't go into those questions,

442 00:17:07.890  $\rightarrow 00:17:10.850$  but this work is really just beginning for us.

443 00:17:10.850 --> 00:17:13.320 Bringing it now back to IDH1, so some of you know

444 00:17:13.320 --> 00:17:16.590 some of the adult midline supratentorial gliomas

445 00:17:16.590 --> 00:17:18.290 have IDH mutations.

446 $00{:}17{:}18.290 \dashrightarrow 00{:}17{:}20.560$  And there's a really an intriguing leak, link

447 00:17:20.560 --> 00:17:22.110 between PPM1D and IDH1.

448 00:17:22.110 --> 00:17:24.900 I alluded to this earlier from the Dan Cahill work

449 00:17:24.900 --> 00:17:26.860 that actually prompted us to serendipitously 450 00:17:26.860 --> 00:17:29.150 sort of make this discovery.

451 00:17:29.150 --> 00:17:31.440 And what, what Dan and colleagues actually found was

452 00:17:31.440 --> 00:17:33.640 similarly in IDH mutants as well,

 $453~00{:}17{:}33.640$  -->  $00{:}17{:}36.520$  they silence NAPRT leading to an NAD depletion.

454 00:17:36.520 --> 00:17:39.330 So we don't understand why adult and pediatric tumors

 $455\ 00:17:39.330 \longrightarrow 00:17:42.670$  with these mutations are silencing

 $456\ 00:17:42.670 \longrightarrow 00:17:44.880$  this pathway, but there's clearly a theme

 $457\ 00{:}17{:}44.880$  -->  $00{:}17{:}49.880$  across all age groups for these tumors for NAD depletion.

458 00:17:50.160 --> 00:17:52.000 So in the last just 5 minutes or so,

 $459\ 00:17:52.000 \longrightarrow 00:17:53.490$  I'll tell you about what we're doing to get this  $460\ 00:17:53.490 \longrightarrow 00:17:55.000$  into the clinic.

461 00:17:55.000 --> 00:17:56.670 So as many of you know we are very interested 462 00:17:56.670 --> 00:17:58.410 in trying to drive some of the work that we do

 $463\ 00:17:58.410 \longrightarrow 00:18:00.300$  into patients as soon as possible.

464 00:18:00.300 --> 00:18:02.690 And this is work that I think

 $465\ 00{:}18{:}02.690$  -->  $00{:}18{:}04.380$  many of you seen us present, and this is work  $466\ 00{:}18{:}04.380$  -->  $00{:}18{:}07.276$  from the Glazer Lab, Stephanie Halene's lab, Morokinaw,

467 00:18:07.276  $-\!>$  00:18:09.820 and my laboratory, essentially mapping out

468 00:18:09.820 --> 00:18:11.690 this on<br/>cometabolite-induced brachinist

 $469\ 00:18:11.690 \longrightarrow 00:18:13.430$  that leads to NAPRT sensitivity.

 $470\ 00:18:13.430 \longrightarrow 00:18:14.710$  And so we've done this before,

 $471\ 00:18:14.710 \longrightarrow 00:18:16.430$  and we've been able to translate this work

 $472\ 00:18:16.430 \longrightarrow 00:18:18.480$  into multiple clinical trials shown here.

473 00:18:18.480 --> 00:18:20.690 And really a testament to the cancer center,

474 00:18:20.690 --> 00:18:23.720 namely folks like, uh, Pat Lorusso, Paul Eder,

475 00:18:23.720 --> 00:18:26.600 Asher Marks, Toma Tebaldi, and again Stephanie Halene

 $476\ 00:18:26.600 \longrightarrow 00:18:30.200$  to really drive this into our patients.

477 00:18:30.200 --> 00:18:33.270 So the questions for this were how we're going to get this

478 00:18:33.270 --> 00:18:37.560 into the clinic, recognizing some of these huge caveats

479 00:18:37.560 --> 00:18:39.900 that I'm going spend the last few minutes on. 480 00:18:39.900 --> 00:18:41.710 So first of all, there are a number of barriers 481 00:18:41.710 --> 00:18:45.470 to a systemic NAMPT inhibitor trial, uh, in

 $482\ 00:18:45.470 \longrightarrow 00:18:47.010$  that we'll touch upon in a moment.

DIPG

483 00:18:47.010 --> 00:18:49.010 We would love to consider combinations

 $484\ 00:18:49.010 -> 00:18:50.700$  with both radiation and chemotherapy

485 00:18:50.700 --> 00:18:52.980 because we don't think monotherapy for any of these,

486 00:18:52.980 --> 00:18:55.510 these aggressive gliomas is going to be sufficient.

487 00:18:55.510 --> 00:18:58.260 And I'll tell you a little bit about some surprising

488 00:18:58.260 --> 00:19:00.480 results about the blood brain barrier penetration

 $489\ 00:19:00.480 \longrightarrow 00:19:02.600$  of some of the drugs that are out there.

490 00:19:02.600 --> 00:19:04.957 So just a few, uh, few points on the first 491 00:19:04.957 --> 00:19:07.370 the first question.

 $492\ 00{:}19{:}07{.}370 \dashrightarrow 00{:}19{:}10{.}580$  So, as I mentioned, multiple NAMPT inhibitor trials

493 00:19:10.580 --> 00:19:12.790 have been initiated and closed.

494 00:19:12.790 --> 00:19:14.854 Most of them ended with lack of efficacy,

495 00:19:14.854  $\rightarrow$  00:19:17.950 and pretty significant doxylamine toxicity.

496 00:19:17.950 --> 00:19:20.540 A lot of folks would say that the

497 00:19:20.540 --> 00:19:23.210 lack of efficacy was simply that these were solid tumor

 $498\ 00:19:23.210 \longrightarrow 00:19:24.780$  Phase 1 trials with no biomarkers.

 $499\ 00:19:24.780 \longrightarrow 00:19:27.630$  They were not trying to find for any specific

 $500\ 00:19:27.630 \longrightarrow 00:19:29.660$  biomarker that could confer sensitivity.

 $501\ 00:19:29.660 \longrightarrow 00:19:32.527$  And the liabilities in particular were

 $502\ 00:19:32.527 \longrightarrow 00:19:34.620$  hemologic and retinal toxicity

503 00:19:34.620 --> 00:19:36.950 which have really spooked a lot of folks that are,

504 00:19:36.950 --> 00:19:39.760 are developing NAMPT inhibitors at the moment,

 $505\ 00:19:39.760 \longrightarrow 00:19:41.150$  and they've shelved them.

50600:19:41.150 --> 00:19:43.460 This is just one paper to show you an example of,

 $507\ 00:19:43.460 \longrightarrow 00:19:45.260$  of this finding.

 $508\ 00:19:45.260 \longrightarrow 00:19:46.830$  So, in parallel to that,

50900:19:46.830 --> 00:19:50.440 we'd love to explore the concept of combining this

510 00:19:50.440 --> 00:19:52.810 with other clinically relevant regimens for glioma,

511 00:19:52.810 --> 00:19:54.280 namely DIPG.

 $512\ 00{:}19{:}54{.}280$  -->  $00{:}19{:}56{.}510$  And it turns out as many of you know in the audience here,

513 00:19:56.510 --> 00:19:59.170 tem<br/>ozolomide is a mainstay of brain tumor treatment.

514 00:19:59.170 --> 00:20:01.500 And temozolomide itself actually has been shown

515 00:20:01.500 --> 00:20:04.960 to cause an NAD depletion by metabolic stress.

516 00:20:04.960 --> 00:20:06.940 In parallel, what about things like radiation,

517 00:20:06.940 --> 00:20:10.000 another mainstay for DIPG and other gliomas?

518 00:20:10.000 --> 00:20:12.150 And I do apologize for I rat out colleagues I know

 $519\ 00:20:12.150 \longrightarrow 00:20:14.090$  to quote a paper from 1978.

520 00:20:14.090 --> 00:20:16.836 I promise I'm going to get a more recent one.

521 00:20:16.836 --> 00:20:18.210 But it turns out that radiation actually depletes

522 00:20:18.210 --> 00:20:19.460 NAD levels as well.

 $523\ 00:20:19.460 \longrightarrow 00:20:21.270$  And so where am I going with this?

 $524\ 00:20:21.270 \longrightarrow 00:20:23.010$  We, we have now NAMPT inhibitors,

525 00:20:23.010 --> 00:20:25.387 possibly radiation temodar - those are, that's like the,

 $526\ 00:20:25.387 \longrightarrow 00:20:27.242$  the stupe trial plus NAMPT inhibitor -

527 00:20:27.242 --> 00:20:30.410 so an opportunity for what I would call trimodality

 $528\ 00:20:30.410 \longrightarrow 00:20:31.770$  synergy with NAMPT inhibitors.

529 00:20:31.770 --> 00:20:34.200 So we're really excited about possibly incorporating

 $530\ 00:20:34.200 \longrightarrow 00:20:37.070$  these modalities into a future clinical trial.

 $531\ 00:20:37.070 \longrightarrow 00:20:37.920$  So the last little point,

 $532\ 00:20:37.920 \longrightarrow 00:20:39.500$  again I just want to give you a flavor for this  $533\ 00:20:39.500 \longrightarrow 00:20:40.333$  because of time.

 $534\ 00:20:40.333 \longrightarrow 00:20:41.560$  There's a lot more to it.

 $535\ 00:20:41.560 \longrightarrow 00:20:43.780$  What about CNS penetration?

 $536\ 00:20:43.780 \longrightarrow 00:20:46.003$  So, one thing we learn is that your drug is no,

537 00:20:46.003 --> 00:20:48.783 no better than how well it can get into the blood,

538 00:20:48.783 --> 00:20:51.520 past the blood brain barrier for glioma trials.

539 00:20:51.520 --> 00:20:53.330 Turns out that most NAMPT inhibitors

540 00:20:53.330 - 00:20:55.070 are CNS impermeable.

541 00:20:55.070  $\rightarrow$  00:20:57.210 The ones that are permeable actually have

542 00:20:57.210 --> 00:20:59.700 that retina toxicity that I mentioned earlier.

 $543\ 00:20:59.700 \longrightarrow 00:21:01.600$  So this is a bit of a conundrum.

544 00:21:01.600 --> 00:21:03.580 And so one thing that we're interested in looking at

545 00:21:03.580 --> 00:21:04.970 is Convection Enhanced Delivery.

546 00:21:04.970 --> 00:21:06.920 Some of you may this, may know of this approach

547 00:21:06.920 --> 00:21:09.980 where you directly inject a drug into the brainstem

548 00:21:09.980 --> 00:21:12.670 or into the brain to by<br/>pass the blood brain barrier.

549 00:21:12.670 --> 00:21:15.220 Folks like Joe Piepmeier and colleagues, uh, have p -

 $550\ 00:21:15.220 \longrightarrow 00:21:16.970$  have done pioneering work in this field.

 $551\ 00:21:16.970 \longrightarrow 00:21:18.610$  And believe it or not, this is actually now,

 $552\ 00:21:18.610 \longrightarrow 00:21:19.560$  now quite common.

553 00:21:19.560 --> 00:21:22.880 There's probably about 7 or 8 trials in kids and adults

 $554\ 00:21:22.880 \longrightarrow 00:21:25.520$  testing CED of novel agents.

 $555\ 00:21:25.520 \longrightarrow 00:21:27.360$  Uh, we would argue that this is a great idea,

556 00:21:27.360 --> 00:21:29.880 but we know within a few hours those drugs you inject,

557 00:21:29.880 --> 00:21:31.210 they wash right away.

558 00:21:31.210 --> 00:21:33.739 Um, and so if the way to encapsulate those drugs

559 00:21:33.739 --> 00:21:36.620 in some sort of particle, i.e. nano-particle,

560 00:21:36.620 --> 00:21:39.700 we could then find a way to prolong, uh, the deliv-

561 00:21:39.700  $\rightarrow$  00:21:42.280 the drug delivery and exposure in the tumor.

 $562\ 00:21:42.280 \longrightarrow 00:21:43.510$  So who could we got to for that?

563 00:21:43.510 --> 00:21:45.090 Well, of course we could go right across the street

564 00:21:45.090 --> 00:21:45.923 to Mark Saltzman.

565 00:21:45.923 --> 00:21:48.790 And Mark and Jianbing Zhou and folks have,

566 00:21:48.790 --> 00:21:50.110 have really done pioneering work

567 00:21:50.110 --> 00:21:53.732 in developing brain penetrating PEG and related

 $568\ 00:21:53.732 \longrightarrow 00:21:56.580$  nano-particles and have shown in

569 00:21:56.580 --> 00:21:59.430 some really seminal papers including this one in PNAS,

570 00:21:59.430 --> 00:22:02.410 that you could use them to treat gliomablastoma.

571 00:22:02.410 --> 00:22:03.958 So we've been working with Mark for quite some time.

 $572\ 00:22:03.958 \longrightarrow 00:22:06.530$  So some of you know over the last couple years

573 00:22:06.530 --> 00:22:08.910 we've had a very, a long fruitful collaboration.

 $574\ 00:22:08.910 \longrightarrow 00:22:10.660$  We've actually shown by proof of concept

575 00:22:10.660  $\rightarrow 00:22:12.780$  that we could take DNA repair inhibitors,

576 00:22:12.780 --> 00:22:15.190 like ATR inhibitors, uh, and encapsulate them

577 00:22:15.190 --> 00:22:17.430 in nano-particles and use them to treat, gliomas.

578 00:22:17.430 --> 00:22:20.620 And this is just one of our papers that came out recently.

579 00:22:20.620 --> 00:22:22.360 So that's actually exactly what we're doing now

 $580\ 00:22:22.360 \longrightarrow 00:22:23.193$  for NAMPT inhibitors.

581 00:22:23.193 --> 00:22:26.970 And this is actually a YCC co-pilot grant

582 00:22:26.970 --> 00:22:29.280 looking at whether we can capsulate NAMPT inhibitors

 $583\ 00:22:29.280 \longrightarrow 00:22:30.490$  in nano-particles.

584 00:22:30.490 --> 00:22:32.810 And this is work from Yazhe Wang and Jason Breckta,

585 00:22:32.810 --> 00:22:34.990 radunct resident in my laboratory showing that

586 00:22:34.990 --> 00:22:37.680 yes, we can and that these particles effectively can

587 00:22:37.680 --> 00:22:40.763 release drug and actually deplete NAD

 $588\ 00:22:40.763 \longrightarrow 00:22:42.520$  in this setting.

 $589\ 00{:}22{:}42.520$  -->  $00{:}22{:}44.860$  So just to wrap up here in the last 2 minutes.

590 00:22:44.860 --> 00:22:47.570 So, we really are firm believers that

591 00:22:47.570  $\rightarrow 00:22:49.180$  metabolic vulnerabilities can be exploited

 $592\ 00:22:49.180 \longrightarrow 00:22:50.790$  in both adult and pediatric gliomas.

 $593\ 00:22:50.790 \longrightarrow 00:22:52.550$  We've shown this for IDH in the adults,

594 00:22:52.550 --> 00:22:54.810 and now we're showing for PPM1D in the kids.

 $595\ 00:22:54.810 \longrightarrow 00:22:57.317$  We believe that just like IDH,

596 00:22:57.317 --> 00:23:00.173 and we're trying to translate this into the clinic.

 $597\ 00:23:00.173 \longrightarrow 00:23:03.940$  We're really falling up as fast as we can

 $598\ 00:23:03.940 \longrightarrow 00:23:05.900$  to understand why PPM1D mutations

599 00:23:05.900 --> 00:23:07.900 are inducing NAPRT silencing.

 $600\;00{:}23{:}07{.}900$  -->  $00{:}23{:}10.810$  And, we do believe that there's an opportunity  $601\;00{:}23{:}10.810$  -->  $00{:}23{:}13.900$  here to take existing treatments like radiation and temodar

 $602\ 00:23:13.900$  --> 00:23:16.050 and bring in NAMPT inhibitors into the fray.

603 00:23:16.050 --> 00:23:18.270 And we're very actively exploring

604~00:23:18.270 --> 00:23:20.250 whether CED and nano-particles may address 605~00:23:20.250 --> 00:23:22.610 some of the issues that I've talked about earlier.

 $606\ 00:23:22.610 \longrightarrow 00:23:24.130$  So with that I'll just wrap up.

 $607~00{:}23{:}24.130 \dashrightarrow 00{:}23{:}26.370$  I'll thank all the folks that did the work

60800:23:26.370 $\operatorname{-->}$ 00:23:28.890 in the laboratory, and all of them are shown here

609 00:23:28.890 --> 00:23:30.280 at our recent retreat.

610 00:23:30.280 --> 00:23:31.160 Nate has moved on.

611 00:23:31.160 --> 00:23:33.360 He's now our first, first grad student,

 $612\ 00:23:33.360 \longrightarrow 00:23:35.477$  and now a post-doc at the NCI.

613 00:23:35.477 --> 00:23:37.160 And of course I'd like to thank the folks that

 $614\ 00:23:37.160 \longrightarrow 00:23:38.250$  fund this work as well.

61500:23:38.250 --> 00:23:39.288 And we have time for a few questions.

 $616\ 00:23:39.288 \longrightarrow 00:23:40.121$  (applause)