

1
00:00:00,000 --> 00:00:08,220
it will called calcein and this is a

2
00:00:02,638 --> 00:00:10,259
relatively impermeable molecule with 363

3
00:00:08,220 --> 00:00:13,500
Dalton the molecular weight is similar

4
00:00:10,259 --> 00:00:15,269
to typical chemotherapy and I'll just

5
00:00:13,500 --> 00:00:17,789
direct you through this these are

6
00:00:15,269 --> 00:00:20,429
microscopic images 10x looking at for

7
00:00:17,789 --> 00:00:22,230
tumor cells here GBM tumor cells and

8
00:00:20,429 --> 00:00:25,198
these mrs. control condition with no

9
00:00:22,230 --> 00:00:29,219
room for sound and just by looking why

10
00:00:25,199 --> 00:00:32,429
light microscopy and if we go across

11
00:00:29,219 --> 00:00:34,320
this is the looking at the fluorescent

12
00:00:32,429 --> 00:00:36,780
image of these same cells so you can see

13
00:00:34,320 --> 00:00:38,488
these four there's a little bit of the

14
00:00:36,780 --> 00:00:40,770
fluorescent dye tech passes into these

15
00:00:38,488 --> 00:00:42,899
cells under control conditions but when

16
00:00:40,770 --> 00:00:44,520
we apply infrasound the intensity of

17
00:00:42,899 --> 00:00:47,009
this fluorescence increases and so you

18
00:00:44,520 --> 00:00:48,930
can see that it's increasing the uptake

19
00:00:47,009 --> 00:00:51,089
of this fluorescent dye unity cells

20
00:00:48,929 --> 00:00:55,409
indicating the membrane is being more

21
00:00:51,090 --> 00:01:00,329
permanent so this is one experiment this

22
00:00:55,409 --> 00:01:02,339
is a histogram using a flow cytometers

23
00:01:00,329 --> 00:01:05,700
to be to get more quantitative measure

24
00:01:02,340 --> 00:01:10,640
of that so basically this is looking at

25
00:01:05,700 --> 00:01:16,049
10,000 events of measuring these

26
00:01:10,640 --> 00:01:17,519
increases so here is 10,000 events with

27
00:01:16,049 --> 00:01:19,430
no in person in the meeting of

28
00:01:17,519 --> 00:01:22,789
fluorescence is here so you see a shift

29

00:01:19,430 --> 00:01:24,930
towards more fluorescence in these cells

30
00:01:22,790 --> 00:01:27,270
so this is very exciting we made it

31
00:01:24,930 --> 00:01:28,829
halfway if we were seeing that we are

32
00:01:27,269 --> 00:01:32,938
seeing an impact on the membrane

33
00:01:28,828 --> 00:01:34,938
permeability with it for some so before

34
00:01:32,938 --> 00:01:37,139
moving to the second hypothesis we

35
00:01:34,938 --> 00:01:40,169
wanted to get a little bit better

36
00:01:37,140 --> 00:01:42,930
on what was coming out of this G machine

37
00:01:40,170 --> 00:01:45,990
because of this to this stage we were

38
00:01:42,930 --> 00:01:47,310
just pressing a button and there was a

39
00:01:45,989 --> 00:01:48,509
little bit light saying that something

40
00:01:47,310 --> 00:01:50,280
was coming out there but we couldn't

41
00:01:48,510 --> 00:01:52,350
hear it and we really just for trusting

42
00:01:50,280 --> 00:01:54,719
that this was making the Chico like

43
00:01:52,349 --> 00:01:58,649

stuff so we we got an engineer to come

44

00:01:54,719 --> 00:02:01,408

in and basically to a spectral analysis

45

00:01:58,650 --> 00:02:05,070

and and so we were pleased that we did

46

00:02:01,409 --> 00:02:07,109

indeed see infrasound that looking

47

00:02:05,069 --> 00:02:11,008

coming out the machine was was in this

48

00:02:07,109 --> 00:02:13,949

usually again the 20 Hertz cut off so we

49

00:02:11,008 --> 00:02:19,469

saw it here between seven and 15 with

50

00:02:13,949 --> 00:02:23,579

some peaks in between so we we forged

51

00:02:19,469 --> 00:02:26,400

ahead oh wait before we first add then

52

00:02:23,580 --> 00:02:30,510

there's 172 that to the chico machine

53

00:02:26,400 --> 00:02:31,890

and let me go back and you can see some

54

00:02:30,509 --> 00:02:33,599

of these peaks and there was this

55

00:02:31,889 --> 00:02:38,129

difference is it was basically get

56

00:02:33,599 --> 00:02:39,599

dynamic frequency and also the richard

57

00:02:38,129 --> 00:02:41,129

lee the scientific director at the

58
00:02:39,599 --> 00:02:43,289
Institute that produces the machine has

59
00:02:41,129 --> 00:02:45,419
spent some time talking to him they feel

60
00:02:43,289 --> 00:02:49,019
very strongly the dynamic component of

61
00:02:45,419 --> 00:02:53,369
this is very important to to protect

62
00:02:49,019 --> 00:02:54,450
against biological adaptation so we

63
00:02:53,370 --> 00:02:56,640
thought well this is really fascinating

64
00:02:54,449 --> 00:02:58,078
and then you know what before we move

65
00:02:56,639 --> 00:03:00,089
forward we wanted to be sure that we

66
00:02:58,079 --> 00:03:03,030
were going to be applying there the best

67
00:03:00,090 --> 00:03:06,360
kind of genome a half site that's kind

68
00:03:03,030 --> 00:03:09,269
of a infrasound so we wouldn't test that

69
00:03:06,360 --> 00:03:11,160
so we made our own g gold generator our

70
00:03:09,269 --> 00:03:15,270
infrasound generator

71
00:03:11,159 --> 00:03:17,400
and I am going to just get you to focus

72
00:03:15,270 --> 00:03:20,189
just on this cluster this this is a

73
00:03:17,400 --> 00:03:22,110
box-and-whisker plot and just to orient

74
00:03:20,189 --> 00:03:24,090
you to it we'll just stick with this

75
00:03:22,110 --> 00:03:25,650
cluster right here it's going to be you

76
00:03:24,090 --> 00:03:27,539
know we have data from four different

77
00:03:25,650 --> 00:03:29,520
human cell lines that are derived from

78
00:03:27,539 --> 00:03:33,659
four different GBM tumors to look for

79
00:03:29,520 --> 00:03:36,060
the generalizability of the results but

80
00:03:33,659 --> 00:03:41,219
just if we look at just one tumor here

81
00:03:36,060 --> 00:03:44,250
u87 the results of this box are from the

82
00:03:41,219 --> 00:03:46,289
dynamic output of the Chico machine and

83
00:03:44,250 --> 00:03:49,550
here are the results from our infrasonic

84
00:03:46,289 --> 00:03:53,370
generator at 8.5 just a single frequency

85
00:03:49,550 --> 00:03:56,280
11.6 and 15 now if you're not familiar

86

00:03:53,370 --> 00:03:58,740
with box and whisker plots there they're

87
00:03:56,280 --> 00:04:02,580
meant to give a depiction of all the

88
00:03:58,740 --> 00:04:06,120
data and basically that the mean is

89
00:04:02,580 --> 00:04:09,180
shown I know that it's convergence of

90
00:04:06,120 --> 00:04:12,270
the hourglass shape and the top of the

91
00:04:09,180 --> 00:04:14,400
box will be the 70 75th percentile than

92
00:04:12,270 --> 00:04:15,750
twenty fifty percent on the bottom the

93
00:04:14,400 --> 00:04:17,519
top of the whiskers are ninety-eight

94
00:04:15,750 --> 00:04:20,459
percent I on the bottom is that two

95
00:04:17,519 --> 00:04:24,149
percent and any outliers any and every

96
00:04:20,459 --> 00:04:26,850
outlier are shown with a cross and and

97
00:04:24,149 --> 00:04:28,439
just to take away from minutes you want

98
00:04:26,850 --> 00:04:31,169
to look for the boxes that don't overlap

99
00:04:28,439 --> 00:04:33,329
and and we really found that most of our

100
00:04:31,168 --> 00:04:36,539

boxes did overlap there were occasions

101

00:04:33,329 --> 00:04:38,909

where where we would get it looks like

102

00:04:36,540 --> 00:04:40,590

an effect but the overall message from

103

00:04:38,910 --> 00:04:43,500

this that we found is that for this

104

00:04:40,589 --> 00:04:45,959

outcome for membrane permeability with

105

00:04:43,500 --> 00:04:50,370

the infrasonic exposure we could pretty

106

00:04:45,959 --> 00:04:52,680

much say that we get we get this

107

00:04:50,370 --> 00:04:54,990

increase in in the fluorescent uptake

108

00:04:52,680 --> 00:04:57,150

whether we use the dynamic frequency or

109

00:04:54,990 --> 00:05:01,410

the single frequency another thing to

110

00:04:57,149 --> 00:05:03,810

note about in some cases the box is not

111

00:05:01,410 --> 00:05:05,760

overlapping is that for the different

112

00:05:03,810 --> 00:05:07,500

cell types there's a look there can be

113

00:05:05,759 --> 00:05:12,779

some differences so some of the reaction

114

00:05:07,500 --> 00:05:14,759

is it's not completely generalizable so

115
00:05:12,779 --> 00:05:15,709
now we move to hypothesis too and we're

116
00:05:14,759 --> 00:05:18,319
looking we're going

117
00:05:15,709 --> 00:05:21,019
the response to a chemotherapy so take

118
00:05:18,319 --> 00:05:23,870
you through the title slowly first we

119
00:05:21,019 --> 00:05:27,109
chose cisplatin very common chemotherapy

120
00:05:23,870 --> 00:05:28,910
it's a DNA damaging agent and our

121
00:05:27,110 --> 00:05:31,730
outcome measure with a pitocin and this

122
00:05:28,910 --> 00:05:33,740
is a its program cell death it's a very

123
00:05:31,730 --> 00:05:37,310
common outcome measure in cancer

124
00:05:33,740 --> 00:05:38,840
research and it's important because it's

125
00:05:37,310 --> 00:05:41,839
an important outcome for cancer because

126
00:05:38,839 --> 00:05:44,839
you said cell basically undergoing cell

127
00:05:41,839 --> 00:05:46,039
suicide rather than necrosis which can

128
00:05:44,839 --> 00:05:48,919
lead to inflammation and be more

129
00:05:46,040 --> 00:05:50,780
problematic so a good chemotherapy will

130
00:05:48,920 --> 00:05:52,939
induce apoptosis so that catch ourselves

131
00:05:50,779 --> 00:05:57,979
were just nice you know packaged up in

132
00:05:52,939 --> 00:06:00,410
and go away so to look at a pitocin we

133
00:05:57,980 --> 00:06:02,840
we stained we had two different stains

134
00:06:00,410 --> 00:06:05,420
and these the results again of a flow

135
00:06:02,839 --> 00:06:08,299
cytometer there are four panels for four

136
00:06:05,420 --> 00:06:09,890
different conditions so we'll get you

137
00:06:08,300 --> 00:06:13,579
know we can focus up here just to orient

138
00:06:09,889 --> 00:06:15,769
you to the craft therefore this is a

139
00:06:13,579 --> 00:06:18,529
control condition with no infrasound and

140
00:06:15,769 --> 00:06:20,359
no cisplatin so you can see that the

141
00:06:18,529 --> 00:06:22,129
healthy cells this is where they live on

142
00:06:20,360 --> 00:06:25,250
this kind of a plot and this lower left

143

00:06:22,129 --> 00:06:28,459
quadrant the end of you because they

144
00:06:25,250 --> 00:06:31,279
don't stain for annexin 5 and this is a

145
00:06:28,459 --> 00:06:33,649
stain that will pick up whenever a

146
00:06:31,279 --> 00:06:36,079
particular lipid will flip its

147
00:06:33,649 --> 00:06:38,419
orientation on the on the membrane and

148
00:06:36,079 --> 00:06:41,539
it's an indicator of the early stages of

149
00:06:38,420 --> 00:06:45,410
a pitocin or programmed cell death so

150
00:06:41,540 --> 00:06:48,140
these healthy cells do not move along

151
00:06:45,410 --> 00:06:50,090
the x-axis because they're not they

152
00:06:48,139 --> 00:06:51,949
don't have this flipping of a membrane

153
00:06:50,089 --> 00:06:56,449
that indicates they've kind of the early

154
00:06:51,949 --> 00:06:58,399
stages of AP ptosis this axis measures

155
00:06:56,449 --> 00:07:00,500
propidium iodide which is a vital dye

156
00:06:58,399 --> 00:07:03,469
which will be allowed into the cell at a

157
00:07:00,500 --> 00:07:05,720

late stage of of cell death regardless

158

00:07:03,470 --> 00:07:07,820

of whether it's a puto sasur not so if

159

00:07:05,720 --> 00:07:10,610

we just look at these quadrants what

160

00:07:07,819 --> 00:07:12,949

you'd see is healthy cells here and then

161

00:07:10,610 --> 00:07:15,740

when we treat them with the key

162

00:07:12,949 --> 00:07:18,259

therapy you see many of the population

163

00:07:15,740 --> 00:07:20,720

this is 24 hours later moving over and

164

00:07:18,259 --> 00:07:23,050

being stained as early a pathetic and

165

00:07:20,720 --> 00:07:25,940

then also moving up into this late stage

166

00:07:23,050 --> 00:07:27,740

cell death so you can see that you know

167

00:07:25,939 --> 00:07:29,509

at 24 hours there were some that were in

168

00:07:27,740 --> 00:07:31,939

the beginning of their program of death

169

00:07:29,509 --> 00:07:33,709

some had already progressed in till late

170

00:07:31,939 --> 00:07:36,219

stage and here's where debris is left

171

00:07:33,709 --> 00:07:39,620

there these are you know completely dead

172
00:07:36,220 --> 00:07:41,810
so this was control and as I've showed

173
00:07:39,620 --> 00:07:43,850
that this is where cisplatin treatment

174
00:07:41,810 --> 00:07:46,879
alone and you can see this death and

175
00:07:43,850 --> 00:07:50,689
then down here this is Empress sound

176
00:07:46,879 --> 00:07:54,500
treatment alone is pretty much

177
00:07:50,689 --> 00:07:56,480
indistinguishable from the the control

178
00:07:54,500 --> 00:07:59,329
condition so there we have the

179
00:07:56,480 --> 00:08:01,910
demonstration of non toxic nature of the

180
00:07:59,329 --> 00:08:03,199
infrasound in this model and we did see

181
00:08:01,910 --> 00:08:05,780
that you can see there are some more

182
00:08:03,199 --> 00:08:08,599
dots over here indicating that on top of

183
00:08:05,779 --> 00:08:11,329
the cisplatin effect we were seeing more

184
00:08:08,600 --> 00:08:13,760
so these are results and again these are

185
00:08:11,329 --> 00:08:15,439
10,000 events these are results from one

186
00:08:13,759 --> 00:08:21,379
experiment comparing the four conditions

187
00:08:15,439 --> 00:08:23,899
and so to look at again four different

188
00:08:21,379 --> 00:08:25,730
cell lines and this represents the data

189
00:08:23,899 --> 00:08:27,739
from three independent experiments for

190
00:08:25,730 --> 00:08:29,689
each of the lines compared to the

191
00:08:27,740 --> 00:08:32,360
control condition so again with

192
00:08:29,689 --> 00:08:34,729
infrasound alone you just saw this kind

193
00:08:32,360 --> 00:08:36,830
of noise not real not a significant

194
00:08:34,729 --> 00:08:39,790
difference in terms of programmed cell

195
00:08:36,830 --> 00:08:42,379
death rate mitosis this is platinum

196
00:08:39,789 --> 00:08:44,269
cause of death and in each case we saw

197
00:08:42,379 --> 00:08:48,379
an increase in the amount of death

198
00:08:44,269 --> 00:08:50,029
caused by the combination so this is

199
00:08:48,379 --> 00:08:51,379
this was a very exciting for us this is

200

00:08:50,029 --> 00:08:54,439
what we were looking for that we could

201
00:08:51,379 --> 00:08:57,649
see an impact in this non toxic agent to

202
00:08:54,440 --> 00:09:02,570
make that chemotherapy more effective so

203
00:08:57,649 --> 00:09:05,360
one more thing we wanted to ask was how

204
00:09:02,570 --> 00:09:07,250
much of the membrane effect how much

205
00:09:05,360 --> 00:09:11,360
could that explain of our cisplatin

206
00:09:07,250 --> 00:09:13,639
effect and this many regression gave us

207
00:09:11,360 --> 00:09:16,870
as you know admittedly it's only three

208
00:09:13,639 --> 00:09:19,909
points but gave us the confidence that

209
00:09:16,870 --> 00:09:22,879
we could talk about a mechanism to

210
00:09:19,909 --> 00:09:25,069
to please the NIH funders and say that

211
00:09:22,879 --> 00:09:28,220
indeed there was a tight correlation

212
00:09:25,070 --> 00:09:30,290
between this and and in addition to

213
00:09:28,220 --> 00:09:32,960
pleasing the funders that also perhaps a

214
00:09:30,289 --> 00:09:35,089

clinical application is that because we

215

00:09:32,960 --> 00:09:37,730

saw some variation and the cells derived

216

00:09:35,090 --> 00:09:40,759

from different tumors this this

217

00:09:37,730 --> 00:09:42,710

indicates it just a simple die just just

218

00:09:40,759 --> 00:09:44,840

looking at the ability of a simple die

219

00:09:42,710 --> 00:09:46,820

to be pushed in the South Amazon might

220

00:09:44,840 --> 00:09:48,769

predict for patient whether this would

221

00:09:46,820 --> 00:09:49,990

be appropriate for them in terms of

222

00:09:48,769 --> 00:09:57,189

assisting in their chemotherapy

223

00:09:49,990 --> 00:10:01,870

treatment so back to external chi gong

224

00:09:57,190 --> 00:10:01,870

you know we try to think think back