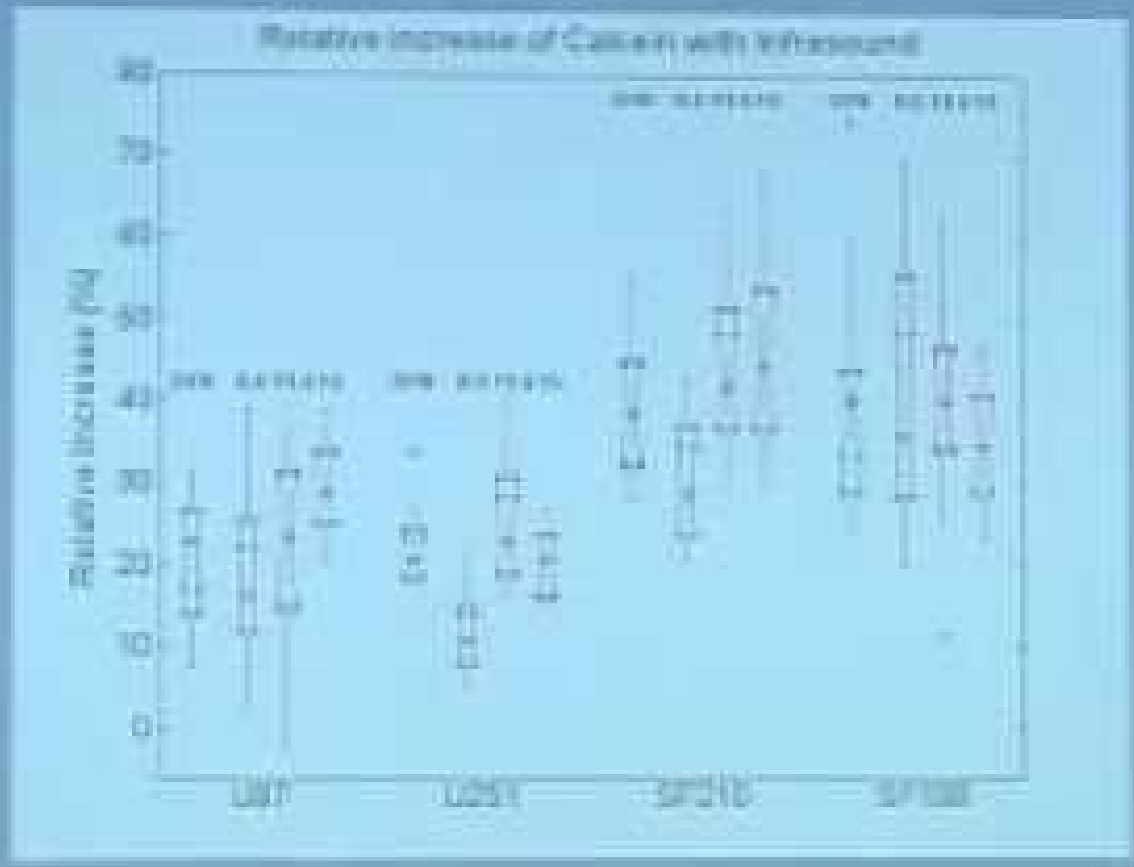
Dynamic vs. Single Frequency



1
00:00:08,210 --> 00:00:02,629
it will called calcein and this is a

2
00:00:10,250 --> 00:00:08,220
relatively impermeable molecule with 363

3
00:00:13,490 --> 00:00:10,260
Dalton the molecular weight is similar

4
00:00:15,259 --> 00:00:13,500
to typical chemotherapy and I'll just

5
00:00:17,779 --> 00:00:15,269
direct you through this these are

6
00:00:20,420 --> 00:00:17,789
microscopic images 10x looking at for

7
00:00:22,220 --> 00:00:20,430
tumor cells here GBM tumor cells and

8
00:00:25,189 --> 00:00:22,230
these mrs. control condition with no

9
00:00:29,210 --> 00:00:25,199
room for sound and just by looking why

10
00:00:32,420 --> 00:00:29,220
light microscopy and if we go across

11
00:00:34,310 --> 00:00:32,430
this is the looking at the fluorescent

12
00:00:36,770 --> 00:00:34,320
image of these same cells so you can see

13
00:00:38,479 --> 00:00:36,780

these four there's a little bit of the

14
00:00:40,760 --> 00:00:38,489
fluorescent dye tech passes into these

15
00:00:42,889 --> 00:00:40,770
cells under control conditions but when

16
00:00:44,510 --> 00:00:42,899
we apply infrasound the intensity of

17
00:00:47,000 --> 00:00:44,520
this fluorescence increases and so you

18
00:00:48,920 --> 00:00:47,010
can see that it's increasing the uptake

19
00:00:51,080 --> 00:00:48,930
of this fluorescent dye unity cells

20
00:00:55,400 --> 00:00:51,090
indicating the membrane is being more

21
00:01:00,319 --> 00:00:55,410
permanent so this is one experiment this

22
00:01:02,330 --> 00:01:00,329
is a histogram using a flow cytometers

23
00:01:05,690 --> 00:01:02,340
to be to get more quantitative measure

24
00:01:10,630 --> 00:01:05,700
of that so basically this is looking at

25
00:01:16,039 --> 00:01:10,640
10,000 events of measuring these

26
00:01:17,510 --> 00:01:16,049
increases so here is 10,000 events with

27
00:01:19,420 --> 00:01:17,520
no in person in the meeting of

28
00:01:22,780 --> 00:01:19,430
fluorescence is here so you see a shift

29
00:01:24,920 --> 00:01:22,790
towards more fluorescence in these cells

30
00:01:27,260 --> 00:01:24,930
so this is very exciting we made it

31
00:01:28,819 --> 00:01:27,270
halfway if we were seeing that we are

32
00:01:32,929 --> 00:01:28,829
seeing an impact on the membrane

33
00:01:34,929 --> 00:01:32,939
permeability with it for some so before

34
00:01:37,130 --> 00:01:34,939
moving to the second hypothesis we

35
00:01:40,160 --> 00:01:37,140
wanted to get a little bit better

36
00:01:42,920 --> 00:01:40,170
on what was coming out of this G machine

37
00:01:45,980 --> 00:01:42,930
because of this to this stage we were

38
00:01:47,300 --> 00:01:45,990

just pressing a button and there was a

39
00:01:48,500 --> 00:01:47,310
little bit light saying that something

40
00:01:50,270 --> 00:01:48,510
was coming out there but we couldn't

41
00:01:52,340 --> 00:01:50,280
hear it and we really just for trusting

42
00:01:54,710 --> 00:01:52,350
that this was making the Chico like

43
00:01:58,640 --> 00:01:54,720
stuff so we we got an engineer to come

44
00:02:01,399 --> 00:01:58,650
in and basically to a spectral analysis

45
00:02:05,060 --> 00:02:01,409
and and so we were pleased that we did

46
00:02:07,100 --> 00:02:05,070
indeed see infrasound that looking

47
00:02:10,999 --> 00:02:07,110
coming out the machine was was in this

48
00:02:13,940 --> 00:02:11,009
usually again the 20 Hertz cut off so we

49
00:02:19,460 --> 00:02:13,950
saw it here between seven and 15 with

50
00:02:23,570 --> 00:02:19,470
some peaks in between so we we forged

51
00:02:26,390 --> 00:02:23,580
ahead oh wait before we first add then

52
00:02:30,500 --> 00:02:26,400
there's 172 that to the chico machine

53
00:02:31,880 --> 00:02:30,510
and let me go back and you can see some

54
00:02:33,590 --> 00:02:31,890
of these peaks and there was this

55
00:02:38,120 --> 00:02:33,600
difference is it was basically get

56
00:02:39,590 --> 00:02:38,130
dynamic frequency and also the richard

57
00:02:41,120 --> 00:02:39,600
lee the scientific director at the

58
00:02:43,280 --> 00:02:41,130
Institute that produces the machine has

59
00:02:45,410 --> 00:02:43,290
spent some time talking to him they feel

60
00:02:49,009 --> 00:02:45,420
very strongly the dynamic component of

61
00:02:53,360 --> 00:02:49,019
this is very important to to protect

62
00:02:54,440 --> 00:02:53,370
against biological adaptation so we

63
00:02:56,630 --> 00:02:54,450

thought well this is really fascinating

64
00:02:58,069 --> 00:02:56,640
and then you know what before we move

65
00:03:00,080 --> 00:02:58,079
forward we wanted to be sure that we

66
00:03:03,020 --> 00:03:00,090
were going to be applying there the best

67
00:03:06,350 --> 00:03:03,030
kind of genome a half site that's kind

68
00:03:09,259 --> 00:03:06,360
of a infrasound so we wouldn't test that

69
00:03:11,150 --> 00:03:09,269
so we made our own g gold generator our

70
00:03:15,260 --> 00:03:11,160
infrasound generator

71
00:03:17,390 --> 00:03:15,270
and I am going to just get you to focus

72
00:03:20,180 --> 00:03:17,400
just on this cluster this this is a

73
00:03:22,100 --> 00:03:20,190
box-and-whisker plot and just to orient

74
00:03:24,080 --> 00:03:22,110
you to it we'll just stick with this

75
00:03:25,640 --> 00:03:24,090
cluster right here it's going to be you

76
00:03:27,530 --> 00:03:25,650
know we have data from four different

77
00:03:29,510 --> 00:03:27,540
human cell lines that are derived from

78
00:03:33,650 --> 00:03:29,520
four different GBM tumors to look for

79
00:03:36,050 --> 00:03:33,660
the generalizability of the results but

80
00:03:41,210 --> 00:03:36,060
just if we look at just one tumor here

81
00:03:44,240 --> 00:03:41,220
u87 the results of this box are from the

82
00:03:46,280 --> 00:03:44,250
dynamic output of the Chico machine and

83
00:03:49,540 --> 00:03:46,290
here are the results from our infrasonic

84
00:03:53,360 --> 00:03:49,550
generator at 8.5 just a single frequency

85
00:03:56,270 --> 00:03:53,370
11.6 and 15 now if you're not familiar

86
00:03:58,730 --> 00:03:56,280
with box and whisker plots there they're

87
00:04:02,570 --> 00:03:58,740
meant to give a depiction of all the

88
00:04:06,110 --> 00:04:02,580

data and basically that the mean is

89
00:04:09,170 --> 00:04:06,120
shown I know that it's convergence of

90
00:04:12,260 --> 00:04:09,180
the hourglass shape and the top of the

91
00:04:14,390 --> 00:04:12,270
box will be the 70 75th percentile than

92
00:04:15,740 --> 00:04:14,400
twenty fifty percent on the bottom the

93
00:04:17,510 --> 00:04:15,750
top of the whiskers are ninety-eight

94
00:04:20,449 --> 00:04:17,520
percent I on the bottom is that two

95
00:04:24,140 --> 00:04:20,459
percent and any outliers any and every

96
00:04:26,840 --> 00:04:24,150
outlier are shown with a cross and and

97
00:04:28,430 --> 00:04:26,850
just to take away from minutes you want

98
00:04:31,159 --> 00:04:28,440
to look for the boxes that don't overlap

99
00:04:33,320 --> 00:04:31,169
and and we really found that most of our

100
00:04:36,530 --> 00:04:33,330
boxes did overlap there were occasions

101
00:04:38,900 --> 00:04:36,540
where where we would get it looks like

102
00:04:40,580 --> 00:04:38,910
an effect but the overall message from

103
00:04:43,490 --> 00:04:40,590
this that we found is that for this

104
00:04:45,950 --> 00:04:43,500
outcome for membrane permeability with

105
00:04:50,360 --> 00:04:45,960
the infrasonic exposure we could pretty

106
00:04:52,670 --> 00:04:50,370
much say that we get we get this

107
00:04:54,980 --> 00:04:52,680
increase in in the fluorescent uptake

108
00:04:57,140 --> 00:04:54,990
whether we use the dynamic frequency or

109
00:05:01,400 --> 00:04:57,150
the single frequency another thing to

110
00:05:03,800 --> 00:05:01,410
note about in some cases the box is not

111
00:05:05,750 --> 00:05:03,810
overlapping is that for the different

112
00:05:07,490 --> 00:05:05,760
cell types there's a look there can be

113
00:05:12,770 --> 00:05:07,500

some differences so some of the reaction

114
00:05:14,750 --> 00:05:12,780
is it's not completely generalizable so

115
00:05:15,700 --> 00:05:14,760
now we move to hypothesis too and we're

116
00:05:18,310 --> 00:05:15,710
looking we're going

117
00:05:21,010 --> 00:05:18,320
the response to a chemotherapy so take

118
00:05:23,860 --> 00:05:21,020
you through the title slowly first we

119
00:05:27,100 --> 00:05:23,870
chose cisplatin very common chemotherapy

120
00:05:28,900 --> 00:05:27,110
it's a DNA damaging agent and our

121
00:05:31,720 --> 00:05:28,910
outcome measure with a pitocin and this

122
00:05:33,730 --> 00:05:31,730
is a its program cell death it's a very

123
00:05:37,300 --> 00:05:33,740
common outcome measure in cancer

124
00:05:38,830 --> 00:05:37,310
research and it's important because it's

125
00:05:41,830 --> 00:05:38,840
an important outcome for cancer because

126
00:05:44,830 --> 00:05:41,840
you said cell basically undergoing cell

127
00:05:46,030 --> 00:05:44,840
suicide rather than necrosis which can

128
00:05:48,910 --> 00:05:46,040
lead to inflammation and be more

129
00:05:50,770 --> 00:05:48,920
problematic so a good chemotherapy will

130
00:05:52,930 --> 00:05:50,780
induce apoptosis so that catch ourselves

131
00:05:57,970 --> 00:05:52,940
were just nice you know packaged up in

132
00:06:00,400 --> 00:05:57,980
and go away so to look at a pitocin we

133
00:06:02,830 --> 00:06:00,410
we stained we had two different stains

134
00:06:05,410 --> 00:06:02,840
and these the results again of a flow

135
00:06:08,290 --> 00:06:05,420
cytometer there are four panels for four

136
00:06:09,880 --> 00:06:08,300
different conditions so we'll get you

137
00:06:13,570 --> 00:06:09,890
know we can focus up here just to orient

138
00:06:15,760 --> 00:06:13,580

you to the craft therefore this is a

139
00:06:18,520 --> 00:06:15,770
control condition with no infrasound and

140
00:06:20,350 --> 00:06:18,530
no cisplatin so you can see that the

141
00:06:22,120 --> 00:06:20,360
healthy cells this is where they live on

142
00:06:25,240 --> 00:06:22,130
this kind of a plot and this lower left

143
00:06:28,450 --> 00:06:25,250
quadrant the end of you because they

144
00:06:31,270 --> 00:06:28,460
don't stain for annexin 5 and this is a

145
00:06:33,640 --> 00:06:31,280
stain that will pick up whenever a

146
00:06:36,070 --> 00:06:33,650
particular lipid will flip its

147
00:06:38,410 --> 00:06:36,080
orientation on the on the membrane and

148
00:06:41,530 --> 00:06:38,420
it's an indicator of the early stages of

149
00:06:45,400 --> 00:06:41,540
a pitocin or programmed cell death so

150
00:06:48,130 --> 00:06:45,410
these healthy cells do not move along

151
00:06:50,080 --> 00:06:48,140
the x-axis because they're not they

152
00:06:51,940 --> 00:06:50,090
don't have this flipping of a membrane

153
00:06:56,440 --> 00:06:51,950
that indicates they've kind of the early

154
00:06:58,390 --> 00:06:56,450
stages of AP ptosis this axis measures

155
00:07:00,490 --> 00:06:58,400
propidium iodide which is a vital dye

156
00:07:03,460 --> 00:07:00,500
which will be allowed into the cell at a

157
00:07:05,710 --> 00:07:03,470
late stage of of cell death regardless

158
00:07:07,810 --> 00:07:05,720
of whether it's a puto sasur not so if

159
00:07:10,600 --> 00:07:07,820
we just look at these quadrants what

160
00:07:12,940 --> 00:07:10,610
you'd see is healthy cells here and then

161
00:07:15,730 --> 00:07:12,950
when we treat them with the key

162
00:07:18,250 --> 00:07:15,740
therapy you see many of the population

163
00:07:20,710 --> 00:07:18,260

this is 24 hours later moving over and

164
00:07:23,040 --> 00:07:20,720
being stained as early a pathetic and

165
00:07:25,930 --> 00:07:23,050
then also moving up into this late stage

166
00:07:27,730 --> 00:07:25,940
cell death so you can see that you know

167
00:07:29,500 --> 00:07:27,740
at 24 hours there were some that were in

168
00:07:31,930 --> 00:07:29,510
the beginning of their program of death

169
00:07:33,700 --> 00:07:31,940
some had already progressed in till late

170
00:07:36,210 --> 00:07:33,710
stage and here's where debris is left

171
00:07:39,610 --> 00:07:36,220
there these are you know completely dead

172
00:07:41,800 --> 00:07:39,620
so this was control and as I've showed

173
00:07:43,840 --> 00:07:41,810
that this is where cisplatin treatment

174
00:07:46,870 --> 00:07:43,850
alone and you can see this death and

175
00:07:50,680 --> 00:07:46,880
then down here this is Empress sound

176
00:07:54,490 --> 00:07:50,690
treatment alone is pretty much

177
00:07:56,470 --> 00:07:54,500
indistinguishable from the the control

178
00:07:59,320 --> 00:07:56,480
condition so there we have the

179
00:08:01,900 --> 00:07:59,330
demonstration of non toxic nature of the

180
00:08:03,190 --> 00:08:01,910
infrasound in this model and we did see

181
00:08:05,770 --> 00:08:03,200
that you can see there are some more

182
00:08:08,590 --> 00:08:05,780
dots over here indicating that on top of

183
00:08:11,320 --> 00:08:08,600
the cisplatin effect we were seeing more

184
00:08:13,750 --> 00:08:11,330
so these are results and again these are

185
00:08:15,430 --> 00:08:13,760
10,000 events these are results from one

186
00:08:21,370 --> 00:08:15,440
experiment comparing the four conditions

187
00:08:23,890 --> 00:08:21,380
and so to look at again four different

188
00:08:25,720 --> 00:08:23,900

cell lines and this represents the data

189
00:08:27,730 --> 00:08:25,730
from three independent experiments for

190
00:08:29,680 --> 00:08:27,740
each of the lines compared to the

191
00:08:32,350 --> 00:08:29,690
control condition so again with

192
00:08:34,719 --> 00:08:32,360
infrasound alone you just saw this kind

193
00:08:36,820 --> 00:08:34,729
of noise not real not a significant

194
00:08:39,780 --> 00:08:36,830
difference in terms of programmed cell

195
00:08:42,370 --> 00:08:39,790
death rate mitosis this is platinum

196
00:08:44,260 --> 00:08:42,380
cause of death and in each case we saw

197
00:08:48,370 --> 00:08:44,270
an increase in the amount of death

198
00:08:50,020 --> 00:08:48,380
caused by the combination so this is

199
00:08:51,370 --> 00:08:50,030
this was a very exciting for us this is

200
00:08:54,430 --> 00:08:51,380
what we were looking for that we could

201
00:08:57,640 --> 00:08:54,440
see an impact in this non toxic agent to

202
00:09:02,560 --> 00:08:57,650
make that chemotherapy more effective so

203
00:09:05,350 --> 00:09:02,570
one more thing we wanted to ask was how

204
00:09:07,240 --> 00:09:05,360
much of the membrane effect how much

205
00:09:11,350 --> 00:09:07,250
could that explain of our cisplatin

206
00:09:13,630 --> 00:09:11,360
effect and this many regression gave us

207
00:09:16,860 --> 00:09:13,640
as you know admittedly it's only three

208
00:09:19,900 --> 00:09:16,870
points but gave us the confidence that

209
00:09:22,870 --> 00:09:19,910
we could talk about a mechanism to

210
00:09:25,060 --> 00:09:22,880
to please the NIH funders and say that

211
00:09:28,210 --> 00:09:25,070
indeed there was a tight correlation

212
00:09:30,280 --> 00:09:28,220
between this and and in addition to

213
00:09:32,950 --> 00:09:30,290

pleasing the funders that also perhaps a

214
00:09:35,080 --> 00:09:32,960
clinical application is that because we

215
00:09:37,720 --> 00:09:35,090
saw some variation and the cells derived

216
00:09:40,750 --> 00:09:37,730
from different tumors this this

217
00:09:42,700 --> 00:09:40,760
indicates it just a simple die just just

218
00:09:44,830 --> 00:09:42,710
looking at the ability of a simple die

219
00:09:46,810 --> 00:09:44,840
to be pushed in the South Amazon might

220
00:09:48,760 --> 00:09:46,820
predict for patient whether this would

221
00:09:49,980 --> 00:09:48,770
be appropriate for them in terms of

222
00:09:57,180 --> 00:09:49,990
assisting in their chemotherapy