Morning. For those of you who don’t know me, I’m Rachel Greenup. I’m Chief of Breast surgery and newly named Co director of the SMILDO Breast Program. And I have the honor today of introducing Dr. Megan King. Doctor King is an associate professor of cell biology and of molecular, cellular and development biology. She’s also the Co leader of radio Biology and Genome Integrity Research program at the Yale Cancer Center and an Associate Cancer Center Director for Basic Science. She did undergrad at Brandeis and
then went on to receive her PhD in Biochemistry and molecular Biophysics from the University of Pennsylvania under the mentorship of Doctor Mark Lemon and went on to get a post doc training with at Rockefeller University where she discovered new mechanisms for the targeting and function of integral membrane proteins. Since founding her own group in 2009, Megan has continued to investigate the broad array of biological functions that are integrated at the nuclear envelope from impacts on DNA repaired to nuclear and cellular mechanisms. She was named a Sarah Scholar in 2011.
and is the recipient of the NIH New Innovator Award and is currently an Allen Distinguished Investigator. She’s been at Yale for 15 years, and we’re excited to hear about her work today. So thank you, Doctor King. Thank you so much. It’s a pleasure to be here. And I think, you know, hearing that bio, it always reminds me of how far I’ve come to what I’m going to be talking about today and how much that is a consequence of the environment at Yale and the interactions that
really have been driven initially
by joining what was on the Radio
biology and genome and radio biology
and radiotherapy research program,
which was connected to me by Patrick Sung,
who’s no longer here.
But he kind of immediately roped
me into that program and then all
of the relationships I made through
particularly with Joanne,
Sweezy and Pat Larusso and really
it’s that transition that is
really spurred everything that
I’m going to talk about today.
And so I’m really appreciative of
that because I think it’s really
going to broaden the scope of fundamental biology, which hopefully you’ll see today about the nuclear envelope is really related to, you know, a chemotherapy approach that’s being broadly used in which we’re hoping could be used and even more context. And so that’s what I’m going to talk about today. And then the surprise to us has been a connection between this and innate immune signaling, and so I really appreciate anyone
00:02:46.236 --> 00:02:48.559 here online now later thoughts
NOTE Confidence: 0.9675821
00:02:48.559 --> 00:02:50.593 on that because there’s so many
NOTE Confidence: 0.9675821
00:02:50.593 --> 00:02:52.830 people at Yale who do have more
NOTE Confidence: 0.9675821
00:02:52.830 --> 00:02:54.820 expertise in that area than we do.
NOTE Confidence: 0.9675821
00:02:54.820 --> 00:02:55.244 OK.
NOTE Confidence: 0.9675821
00:02:55.244 --> 00:02:56.940 So just my disclosure,
NOTE Confidence: 0.9675821
00:02:56.940 --> 00:02:59.156 some of this work is funded through the
NOTE Confidence: 0.9675821
00:02:59.156 --> 00:03:00.580 strategic alliance with AstraZeneca.
NOTE Confidence: 0.539626
00:03:03.150 --> 00:03:06.118 So as as many of you are familiar
NOTE Confidence: 0.539626
00:03:06.118 --> 00:03:09.107 with PARP inhibitors are really the
NOTE Confidence: 0.539626
00:03:09.110 --> 00:03:12.030 canonical example of synthetic lethality.
NOTE Confidence: 0.539626
00:03:12.030 --> 00:03:14.746 And it’s such a powerful concept because
NOTE Confidence: 0.539626
00:03:14.746 --> 00:03:17.381 it really highlights how we might use
NOTE Confidence: 0.539626
00:03:17.381 --> 00:03:19.131 approaches that are really specific
NOTE Confidence: 0.539626
00:03:19.131 --> 00:03:21.613 to tumor cells and otherwise do not
NOTE Confidence: 0.539626
00:03:21.613 --> 00:03:23.854 affect all the normal cells of the body.
And what is you know, fabulous approach, right that that would be. And so the idea is that PARP inhibitors in particular cause single stranded DNA damage to persist or at least that’s one of the mechanisms that we think about as being important here. However, in the consequence of defects and homologous recombination this kind of damage because they have a functional homologous or combination DNA repair mechanism that can act in SNG 2 and repair these breaks. And this leads to cell survival.
and kind of the classic example of pathogenic mutations in the BRCA one and BRCA 2 genes. There's a defect in tolerating this damage and this will lead to cell death, right. And so this is the mechanism where it's the combination of the HR defect on the PARP inhibitor that drives a tumor cell death. So I want to just set the stage for what I'm going to talk about today by reminding you about how P53 works because I'm going to use this framework for thinking about the story.
that I'm going to tell the debt.

So in interface in normal cells, right,

we have when there’s DNA damage,

there is the activation of P53 and

P53 is really this decision point,

It’s both activating mechanisms to

repair that damage, right.

So that the first response of the cell

is try to tolerate and repair this damage,

stall the cell cycle,

fix the genome and then go into mitosis

and and have normal cell growth.

But if this damage is too deleterious,

if it persists,

if it can’t be tolerated,
then this is going to lead to
the stimulation of apoptosis.
And so really this is this combination of repair and then when we can’t repair driving cell death,
however, you know, we know that this is a mechanism that is dysregulated in the vast majority of tumors including those that respond to PARP inhibitors. And so this is not the mechanism, right. So we know we can get the synthetic lethality of PARP inhibitors with HR defects even in the context of dysregulated P53.
So what is this mechanism actually and you might think that we understand this mechanism, but what I’m going to tell you about today is that we don’t and I’m going to focus today disclaimer on the tumor cell intrinsic mechanisms. That is not to negate the fact that there are other roles for the immune system for the tumor microenvironment. But what we know is that in HR deficient cells in a dish PARP inhibitors can cause cell deaths. So we know that there is at least a sufficiency in cells and
culture for a tumor cell intrinsic mechanism of cell death and IT and how do we think about what kind of surveillance mechanisms might be akin to P53 that drive this. So I just want to highlight a few of the challenges that we face in the use of HARP inhibitors because really this is our motivation for the kind of fundamental studies that I'm going to talk about. You know, it's very clear that PARP inhibitors specifically kill HR deficient cells, but we don't understand the cell death mechanism.
As I already highlighted, acquired resistance is a major challenge and it’s really well explored in preclinical models through things like CRISPR screens. But actually the insights from patient samples is really still rather limited and understanding the cell death mechanism that PARP inhibitors precipitate could really help in this area. A major challenge is that we lack a robust biomarker that can tell us that PARP inhibitors are likely to be effective. So this can either be that.
00:07:08.018 --> 00:07:08.777 cells are reconstituted,
NOTE Confidence: 0.531500307142857
00:07:08.780 --> 00:07:10.412 homologous recombination or there
NOTE Confidence: 0.531500307142857
00:07:10.412 --> 00:07:13.258 could be other contexts outside of the
NOTE Confidence: 0.531500307142857
00:07:13.258 --> 00:07:15.238 genetic kind of germline mutations and
NOTE Confidence: 0.531500307142857
00:07:15.238 --> 00:07:17.794 BRCA one and BRCA 2 or even somatic
NOTE Confidence: 0.531500307142857
00:07:17.794 --> 00:07:19.687 mutations where it could be there
NOTE Confidence: 0.531500307142857
00:07:19.687 --> 00:07:22.009 is an HR defect that’s actionable.
NOTE Confidence: 0.531500307142857
00:07:22.010 --> 00:07:23.991 But because we don’t have a biomarker
NOTE Confidence: 0.531500307142857
00:07:23.991 --> 00:07:26.290 for HR status that is at least dynamic,
NOTE Confidence: 0.531500307142857
00:07:26.290 --> 00:07:26.650 right,
NOTE Confidence: 0.531500307142857
00:07:26.650 --> 00:07:28.450 There are kind of sequencing
NOTE Confidence: 0.531500307142857
00:07:28.450 --> 00:07:29.170 based approaches,
NOTE Confidence: 0.531500307142857
00:07:29.170 --> 00:07:31.900 but we don’t have a classic kind
NOTE Confidence: 0.531500307142857
00:07:31.900 --> 00:07:33.070 of pathological straightforward
NOTE Confidence: 0.531500307142857
00:07:33.134 --> 00:07:34.678 psychology kind of approach
NOTE Confidence: 0.531500307142857
00:07:34.678 --> 00:07:36.608 and that’s a real limitation.
And lastly, there's a lot of enthusiasm about combining PARP inhibitors with immune checkpoint blockades and indeed a number of trials that are exploring this. But we don’t actually understand the underlying mechanisms of why those combinations might be effective. And to really understand that, we have to understand how carpenters are working and and This is why we're really interested in the crosstalk. I’ll talk about today with the innate immune system and how that might be contribute to the rationale for
these combinations and might point to what the right approaches are.

So as I said, I'm going to focus on this cell death mechanism in my talk today and to introduce how we've kind of how we've been thinking about this problem. I want to just introduce you to this canonical innate immune surveillance mechanism in which C Gas shown here is a key player. So C gas is an innate immune sensor protein that is in the cytoplasm of cells and it binds to double stranded DNA. And the idea is that it can surveil 4 viruses and bacterial pathogens,
but there’s increasing evidence that C gas is also capable of surveilling self DNA that’s present within cells within eukaryotic cells themselves. So for example, a distranged mitochondria can lead to leaking of mitochondrial DNA into the cytoplasm which can activate C gas. And today I’m going to be talking about how actually the chromosomes or the chromatin or DNA from the nucleus can be exposed and surveilled by C gas. C gas works by when it binds to DNA. Just I’m going to say very clearly, when it binds to naked DNA,
this drives a change and molecules of C gas come together and they produce the second messenger called C gamp. But actually binding of C gas to DNA does not always lead to this response. And so there’s regulation of this that I’ll talk about in more detail in a moment. So just recruiting C gas somewhere does not mean that it’s actually producing this second messenger, but the second messenger is thought to be key to its downstream mechanisms. The recipient of the C gamp signal is sting. Sting is a membrane protein that is key to the canonical signaling pathway that C gas activates.
And that is by driving the phosphorylation of a kinase called TBK one once, its traffic to the Golgi and then this phosphorylates IRF 3 which is a transcription factor that when phosphorylated goes into the nucleus and drives interferon stimulated gene expression. So that’s the kind of canonical pathway. There’s also a non canonical roles in activating NF Kappa B signaling and any of these may in addition to inflammatory genes cause apoptosis. So this could be a mechanism that can drive cell death, although we really don’t understand
this terribly well. In addition, sting is also involved in some other non canonical mechanisms that could also precipitate cell death, which as I mentioned is what I'm going to be focusing on today and part of this actually involves the autophagy mechanisms. There appears to be some autophagy dependent cell death mechanism downstream of sting and this is independent perhaps of this canonical interferon stimulated gene signaling. And so while I'm going to focus kind of on these upstream steps today, we really don't know what the key downstream
steps are in terms of which signaling pathways are going to be most relevant. And so that’s really kind of ongoing work. And I’ll just close this slide by highlighting that actually AC gas is a really ancient protein. It actually goes all the way back to prokaryotes. And so it’s played a role in surveilling foreign DNA long before the innate immune system. And so that kind of makes sense, this idea that it’s actually multiple signaling pathways that lie downstream of C gas activation.
So how do we get thinking about innate immunity? There's abundant evidence in the literature that HR defects on this. In this particular case on the left, we're looking at bracket. In both these cases, we're looking at bracket to knock down models that HR defects are sufficient to trigger an innate immune response and this is a response that's actually further pushed by the addition of PARP inhibitors. So let me just walk you through the example of this data. As I mentioned,
these are BRCA 2 knock down cells.

So with doxycycline we have suppression A BRCA 2 expression and you can see that there’s a gain in IRF 3 phosphorylation which is one of that canonical downstream outcomes of C gas signaling.

And this also leads in this model to Stat 1 phosphorylation. And a similar thing is seen in the in breast cancer cells in this 231 model.

Again, this is a artificial system of the knock down of BRCA 2 with regards to how PARP inhibitors
then synergize with this.

I've just pulled out this data from BRCA 1 deficient, BRCA 1 deficient breast cancer line that’s commonly used in the lab to study a BRCA 1 deficiency and this is now in a xenograft model. So these are actually now xenographs looking at how PARP inhibitors affect interferon stimulated gene expression. And you can see that all of these genes that are downstream of C gas activation are up regulated in the with PARP inhibitor treatment in the xenograft model. So there’s been these observations
NOTE Confidence: 0.5958521
00:13:00.745 --> 00:13:02.816 of innate immune stimulation in
NOTE Confidence: 0.5958521
00:13:02.816 --> 00:13:05.564 the context of HR deficient cells
NOTE Confidence: 0.5958521
00:13:05.564 --> 00:13:07.925 that’s further pushed by PARP
NOTE Confidence: 0.5958521
00:13:07.925 --> 00:13:09.820 inhibitors in a number of cases.
NOTE Confidence: 0.5958521
00:13:09.820 --> 00:13:13.019 But what is the cause of this?
NOTE Confidence: 0.5958521
NOTE Confidence: 0.5958521
00:13:13.305 --> 00:13:14.160 So what the,
NOTE Confidence: 0.5958521
00:13:14.160 --> 00:13:15.300 what the signal is,
NOTE Confidence: 0.5958521
00:13:15.300 --> 00:13:18.092 How we go from HR deficiency to innate
NOTE Confidence: 0.5958521
00:13:18.092 --> 00:13:20.380 immune signaling has been really unclear.
NOTE Confidence: 0.5958521
00:13:20.380 --> 00:13:22.151 One other thing that I want to
NOTE Confidence: 0.5958521
00:13:22.151 --> 00:13:23.872 just alert you to is that when
NOTE Confidence: 0.5958521
00:13:23.872 --> 00:13:25.620 there is an HR defect in cells,
NOTE Confidence: 0.5958521
00:13:25.620 --> 00:13:27.640 one of the consequences is
NOTE Confidence: 0.5958521
00:13:27.640 --> 00:13:29.256 that we accumulate cells,
accumulate mitotic errors.

So this is just one paper I’ve pulled out from Steve West, actually from more than a decade or probably more than 15 years ago now where it’s been recognized for a long time. If there are challenges in maintaining integrity of the genome, then in mitosis you have these intermediates that lead to persistent bridges of DNA and DNA breaks and these kind of breakage, fusion breakage cycles that can actually be precipitated by an HR defect, by radiation, by taxol treatments. You can arrive at these kind
NOTE Confidence: 0.5958521
00:14:01.730 --> 00:14:02.960 of structures in many ways.
NOTE Confidence: 0.5958521
00:14:02.960 --> 00:14:04.418 But I would say HR deficiency
NOTE Confidence: 0.5958521
00:14:04.418 --> 00:14:06.406 is not the way that most people
NOTE Confidence: 0.5958521
00:14:06.406 --> 00:14:08.006 have thought about arriving at
NOTE Confidence: 0.5958521
00:14:08.006 --> 00:14:09.480 these kind of structures.
NOTE Confidence: 0.7051682
00:14:11.530 --> 00:14:13.245 I also just want to remind you,
NOTE Confidence: 0.7051682
00:14:13.250 --> 00:14:15.410 'cause I'm a cell biologist,
NOTE Confidence: 0.7051682
00:14:15.410 --> 00:14:18.410 'cause I'm a cell biologist,
NOTE Confidence: 0.7051682
00:14:18.410 --> 00:14:20.432 not only the nuclear envelope,
NOTE Confidence: 0.7051682
00:14:20.432 --> 00:14:22.440 breaks down every cell cycle. OK.
NOTE Confidence: 0.7051682
00:14:22.440 --> 00:14:24.510 So I just wanted to keep this in your
NOTE Confidence: 0.7051682
00:14:24.510 --> 00:14:26.643 mind too as I talk about this because
NOTE Confidence: 0.7051682
00:14:26.650 --> 00:14:29.114 I just told you there’s an innate
NOTE Confidence: 0.7051682
00:14:29.114 --> 00:14:30.822 immune surveillance protein that is
NOTE Confidence: 0.7051682
00:14:30.822 --> 00:14:32.887 looking for DNA and yet every mitosis,
the chromosomes are exposed to the cytoplasm.

So we know that that’s not sufficient to drive an innate immune response.

So we know in mitosis there are mechanisms to down rate,

are a way to shield these chromosomes from actually activating this pathway.

And so these recombination intermediates are interesting in part because they don’t just occur in mitosis,

they persist into the following interface.

And that’s going to be important here because we need to get to the next interphase in order for this innate immune surveillance mechanism to be reactivated.
And indeed, there is also evidence in the literature that for PARP inhibitors to actually induce cell death, cells have to transit through mitosis. This is additional evidence that you know, unlike P53, which as I mentioned is acting an interphase, that it is essential for cells to go through mitosis for PARP inhibitors to actually cause the cell death. This is actually some work again in a xenograph model and the absence of functional bracket, two and cells treated with a laparib and what you can see is kind of these events.
So we have a cell that is likely in G2, it goes into mitosis. You can see this is an anaphase. So there are anaphase bridges here and actually most cells have some degree of entanglement of chromosomes in anaphase that are going to be resolved dynamically. However, if that does not happen, so what happens is that these cells will biochemically come out of mitosis. So they’re back in interface and you can see that because the nucleus is intact again. But what you can see in this cell is you now have a doublet essentially.
00:16:12.550 --> 00:16:12.856 right?

00:16:12.856 --> 00:16:14.998 You have a cell that actually failed

00:16:14.998 --> 00:16:16.929 in cytokinesis and it failed because

00:16:16.929 --> 00:16:18.509 you couldn’t actually generate 2

00:16:18.509 --> 00:16:20.174 cells because there was bridging

00:16:20.174 --> 00:16:21.784 DNA between these two cells.

00:16:21.790 --> 00:16:23.350 But the cell has biochemically

00:16:23.350 --> 00:16:25.492 come back into interface and so we

00:16:25.492 --> 00:16:27.074 can imagine that the innate immune

00:16:27.074 --> 00:16:28.350 system is active again.

00:16:28.350 --> 00:16:29.614 And the question is,

00:16:29.614 --> 00:16:31.927 is this somehow aware of the fact

00:16:31.927 --> 00:16:34.027 that this is a defective mitosis?

00:16:34.030 --> 00:16:35.703 Is there some mechanism to know that

00:16:35.703 --> 00:16:37.429 and that this would ultimately Dr.
the cell death and that’s what we see happening on the right with this chromosome condensation.

I just want to highlight that this is not really new information, so we can go back.

This is from 2001 and there has been long been the understanding that these, the changes in nuclear shape, nuclear atypia which are used all the time by pathologists to diagnose and stays, cancers are tied to these kind of aberrations that I’ve mentioned.

So I just want to you know that they’ve been called many things over time.

What I want to point out is that all
00:17:14.480 --> 00:17:16.998 of these kind of mitotic errors that
00:17:16.998 --> 00:17:18.963 are typically associated with altered
00:17:18.963 --> 00:17:21.270 nuclear shape are all things that we’re
00:17:21.270 --> 00:17:22.750 observing in interphase cells again,
00:17:22.750 --> 00:17:24.458 so not in cells just in mitosis
00:17:24.458 --> 00:17:25.910 that have an anaphase bridge
00:17:25.910 --> 00:17:27.590 but they’re in in interphase.
00:17:27.590 --> 00:17:29.970 So these were called what the structures
00:17:29.970 --> 00:17:32.211 that I just described that you can
00:17:32.211 --> 00:17:34.402 have persistent DNA that then is still
00:17:34.402 --> 00:17:36.208 there as cells reform their nucleus
00:17:36.208 --> 00:17:38.310 and go into the next cell cycle.
00:17:38.310 --> 00:17:39.598 And this, you know,
00:17:39.598 --> 00:17:41.530 25 years ago were called inter
00:17:41.530 --> 00:17:42.630 nuclear strings,
but you can also have micronuclei.

And I just want to point out one of the differences between these two types of structures is that these inter nuclear strings are because of an inability to segregate the chromosomes because the chromosomes are literally entangled and cannot be physically segregated.

Micronuclei are different and that they predominantly arise from lagging chromosomes, acentrosomal chromosome fragments and perhaps extra chromosomal DNA, right.

So they really are a different structure than these two structures.
are actually quite different.

And I’ll come back to that.

The consequence of this can lead to BI nucleation.

That’s what I just showed you in that particular bracket 2 model.

And I won’t really talk about it today, but you can also get nuclear ruptures that happen in interface due to a defect in the nuclear integrity.

But that is not an event that’s tied to mitosis.

So I’m not going to talk more about that today.

OK.
So let me just show you kind of the amazing cell biology that is tied and specifically to these persistent DNA bridges. So here I’m showing you a movie. These are cells that are expressing a nuclear localization signal tagged to a fluorescent protein. So it’s exclusively in the nucleus. And we’re going to look at this cell that is just going through mitosis, if it will. Maybe I’m not allowed to do that while I have the pointer on, Is that possible? Yeah, that’s possible.
NOTE Confidence: 0.9209971
00:19:09.670 --> 00:19:11.289 that is trying to transit mitosis.
NOTE Confidence: 0.9209971
00:19:11.290 --> 00:19:12.730 We’re gonna see it come out of mitosis.
NOTE Confidence: 0.9209971
00:19:12.730 --> 00:19:14.020 These cells are still linked by
NOTE Confidence: 0.9209971
00:19:14.020 --> 00:19:15.649 one of these DNA bridges and you
NOTE Confidence: 0.9209971
00:19:15.649 --> 00:19:17.083 can see there are these flashes,
NOTE Confidence: 0.9209971
00:19:17.090 --> 00:19:18.460 there are these transient ruptures
NOTE Confidence: 0.9209971
00:19:18.460 --> 00:19:20.473 of the nucleus and all the nuclear
NOTE Confidence: 0.9209971
00:19:20.473 --> 00:19:21.943 localization signal will spill out
NOTE Confidence: 0.9209971
00:19:21.943 --> 00:19:23.978 and then there seems to be some repair
NOTE Confidence: 0.9209971
00:19:23.978 --> 00:19:25.822 of that event and then the the
NOTE Confidence: 0.9209971
00:19:25.822 --> 00:19:27.286 protein can start to accumulate again.
NOTE Confidence: 0.9209971
00:19:27.290 --> 00:19:30.076 So it’s kind of these cycles of
NOTE Confidence: 0.9209971
00:19:30.076 --> 00:19:32.490 ruptures and then repair events.
NOTE Confidence: 0.9209971
00:19:32.490 --> 00:19:34.290 So this is just looking in this case,
NOTE Confidence: 0.9209971
00:19:34.290 --> 00:19:37.050 this is actually a model where there’s
NOTE Confidence: 0.9209971
a dicentric chromosome, however one. So one of the questions is what’s the consequence of this innate immune surveillance mechanism when you have one of these ruptures. So these kind of transient ruptures of the nuclear envelopes, right. So the nucleus, we’ve come out of mitosis, it should be intact, but it’s unstable. And so here I’m going to show you similarly cells, but these cells are actually now expressing AC gas that’s tagged and that’s going to be in this panel here. And I just want to again point out this is not just anaphase.
This is far after anaphase.

These cells have this bridge.

They’re trying to break their DNA

and and segregate it, right,

Not break it, but segregate it.

And what I hope you can appreciate

is that late in this movie,

all of a sudden what we see is

that there’s recruitment of sea

gas all over this strand of DNA.

OK. So it’s not something that

happens in mitosis.

It’s far after mitosis.

There is this bridge of DNA the

nuclear was trying to form around it,
but we get these ruptures and see gases recruited and this is a persistent bridge.

I just want to point out you also get this kind of thing to Micronuclei.

Here’s a micronucleus and we can actually see that that micronucleus is intact and then it’s going to rupture and then there’s massive C gas recruitment,

So any of these losses of nuclear integrity, whether it’s one of these persistent Dania bridges or it’s a micronucleus can recruit the C gas protein.

So I’m going to focus today on these DNA bridges and I’m going to just give you the rationale for why that is.
Now, one of them is that actually many perturbations will cause both these DNA bridges and micronuclei. But there’s evidence in the literature that DNA bridges are actually much more potent activators of Segamp production. If you remember, I told you, the recruitment of C gas is not sufficient to activate it to generate high levels of Segamp. You know why might that be? There’s evidence actually that one of the mechanisms that keeps cells from overreacting to its own genome is the fact that nucleosomal or chromatized DNA...
00:21:45.463 --> 00:21:48.292 is a poor stimulator of Segamp production.
NOTE Confidence: 0.27129424
00:21:48.292 --> 00:21:49.870 Whereas naked DNA,
NOTE Confidence: 0.27129424
00:21:49.870 --> 00:21:51.870 what you would have in a virus or a bacteria,
NOTE Confidence: 0.27129424
00:21:51.870 --> 00:21:54.150 is a far more potent activator
NOTE Confidence: 0.27129424
00:21:54.150 --> 00:21:55.670 of C gamp activation.
NOTE Confidence: 0.27129424
00:21:55.670 --> 00:21:57.944 And so this would suggest that
NOTE Confidence: 0.27129424
00:21:57.944 --> 00:22:00.749 really the state of the DNA matters.
NOTE Confidence: 0.27129424
00:22:00.750 --> 00:22:02.806 And what I'm going to argue here is
NOTE Confidence: 0.27129424
00:22:02.806 --> 00:22:04.474 that actually micronuclei for the
NOTE Confidence: 0.27129424
00:22:04.474 --> 00:22:06.309 most part are chromatized substrate.
NOTE Confidence: 0.27129424
00:22:06.310 --> 00:22:07.810 It was a lagging chromosome
NOTE Confidence: 0.27129424
00:22:07.810 --> 00:22:09.310 that formed its own nucleus.
NOTE Confidence: 0.27129424
00:22:09.310 --> 00:22:12.988 It’s unstable but still it’s nucleosomal
NOTE Confidence: 0.27129424
00:22:12.990 --> 00:22:14.766 whereas this DNA in these persistent
NOTE Confidence: 0.27129424
00:22:14.766 --> 00:22:16.869 bridges as you saw in those movies,
NOTE Confidence: 0.27129424
00:22:16.870 --> 00:22:18.868 the DNA is being pulled apart.
And so one of the ideas is that it

there’s so much tension on the DNA

that actually the histones that make

nucleosomes are being evicted and then

the DNA that’s left is naked and that

that is a more potent activator C camp.

And additional evidence from that

for that comes from observations

that Apobac activity it is actually

very high over overstretched DNA

that is present in bridges,

which suggests that it can also become

single stranded and acted on by APOBEC.

Such as additional evidence that

the structure in these in these
persistent DNA bridges is different than what might be in Micronuclei. OK. And then last bit of cell biology before I get into our own data that I need to introduce you to is the idea that like in that NLS movie, there’s also a nuclear envelope repair mechanism that is looking for these breaks in the nuclear envelope and trying to fix it. And this is something that’s been of interest to our group for a long time. So remember as I said, in a normal mitosis, the nuclear envelope has broken down,
the chromosomes are exposed, but they don’t activate the innate immune system. Then we reform the nuclear envelope at mitotic exit. When the nuclear envelope is reformed, you have sheets of endoplasmic reticulum around the chromosomes, but it’s full of holes actually. And those holes are particularly where there are still microtubules from the spindle that are attached to the chromosomes. So there is a machinery that has to come in and fix all these holes.
00:23:40.957 --> 00:23:42.740 at the end of every mitosis.
NOTE Confidence: 0.27129424
00:23:42.740 --> 00:23:45.050 And that machinery is made-up of the
NOTE Confidence: 0.27129424
00:23:45.050 --> 00:23:46.740 components that I’ve shown here.
NOTE Confidence: 0.27129424
00:23:46.740 --> 00:23:48.522 There is an abundant DNA binding
NOTE Confidence: 0.27129424
00:23:48.522 --> 00:23:49.413 protein called bath,
NOTE Confidence: 0.73344946
00:23:49.420 --> 00:23:52.444 not to be confused with the chromatin
NOTE Confidence: 0.73344946
00:23:52.444 --> 00:23:54.998 remodeler bath and this brings in
NOTE Confidence: 0.73344946
00:23:54.998 --> 00:23:57.688 a protein called LEM 2 which is
NOTE Confidence: 0.73344946
00:23:57.688 --> 00:23:59.133 an integral membrane protein and
NOTE Confidence: 0.73344946
00:23:59.133 --> 00:24:00.789 that’s shown here in the cartoon.
NOTE Confidence: 0.73344946
00:24:00.790 --> 00:24:02.806 So this LEM Two is recruited to these
NOTE Confidence: 0.73344946
00:24:02.806 --> 00:24:05.478 holes in the nuclear envelope and LEM Two
NOTE Confidence: 0.73344946
00:24:05.478 --> 00:24:07.830 is an adapter for the escort machinery,
NOTE Confidence: 0.73344946
00:24:07.830 --> 00:24:09.798 particularly CHIM 7 which is a
NOTE Confidence: 0.73344946
00:24:09.798 --> 00:24:11.110 nuclear envelope specific escort.
NOTE Confidence: 0.73344946
00:24:11.110 --> 00:24:13.483 So the escorts are a membrane remodeling
machinery that basically can take a hole in a membrane and they can close it. And so this machinery is recruiting, is recruiting escorts to the nuclear envelope they form these spiral polymers, and you need this to have one nuclear envelope at the end of mitosis. So this is the normal thing that’s always happening. But there’s abundant evidence that this same exact machinery is recruited anytime there’s a defect in nuclear integrity. And so I’m just showing you an example of this here. This is actually where a rupture in
the nuclear envelope has been induced.

And you can see that there’s recruitment of this escort chimp 7 as well as recruitment of sea gas, right.

So one way of thinking about this kind of similar to the P53 story, you can repair DNA or the cell can die and you can give up on things.

We have this machinery that sees a hole in the nuclear envelope. It can try to fix the hole, but if it can’t fix the hole, there’s a surveillance by the innate immune system. And so there’s actually a
competition potentially that’s going on between these factors.
And I’ll show you some evidence for that in a moment.
Right.
So here is,
and I’m just going to lay out why we’ve done the experiments that I’m going
to describe in the rest of the talk.
I’ve already walked through
Interphase and the idea, P 53.
So I just want to make the argument up front for the hypothesis of a similar surveillance mechanism that’s active post mitosis to Surveil,
the integrity of the mitotic process.
So if cells go into mitosis with
under replicated DNA or unresolved DNA repair intermediates,
these are things which we're going to see in an HR deficient cell,
particularly one that's been treated with PARP inhibitors or
chromosomes that are entangled.
This will initially activate mechanisms that try to help segregate these chromosomes.
This involves proteins like the Bloom helicase on the pitch healer case,
Paul Theta, You know,
mediated and joining,
also as other topo isomerases.
But if those repair, you know those attempts to segregate chromosomes fail, then one of the consequences I’ve shown you is that you can have defects in nuclear integrity and now the cell has to kind of decide what to do. So there’s a nuclear envelope repair network And so I showed you this bath LEM two chimp 7 access that as I’ve mentioned our group has worked on for a long time understanding the mechanisms of and that this can promote cell survival and possibly genome integrity.
On the other hand if they’re unable to repair these breaks in the nucleus then this will expose DNA. This can activate C gas and perhaps this is the mechanism of cell death that is tied to mitosis and is tied to these observations of innate immune signaling that occur as a consequence of PARP inhibitors in HR deficient cells.

And I just want to point out that, right, we’re going to push these further if we any time we disrupt the checkpoint, right. So if cells are going into mitosis when they have not repaired their DNA, these are more likely to happen if you have an HR defect and if you...
treat cells with a PARP inhibitor.
The very last thing I'll talk about is, is there a way that we might use this nuclear integrity defects as a biomarker of HR defects or of contacts where PARP inhibitors might be effective. So I'll come back to that at the end. And also might this nuclear envelope repair network be a new target, right. These are factors which actually limit the action potentially of agents that are driving these defects that we're using clinically. OK.
some of the data from our group.

This initial data is using actually an ovarian cancer model, UWB 1280 nines which are a BRCA 1 deficient, HR deficient cell line. And so I'm just showing you an example of what one of these persistent DNA bridges look like. This is. You can think of this as very much as the end point of that movie specifically in HR in this HR deficient line that’s further precipitated by the addition of PARP inhibitors. And so like in that example you can
00:28:18.762 --> 00:28:21.335 see that this bridge which is all
along connecting these two nuclei
00:28:23.350 --> 00:28:25.228 is highly enriched in C gas.
00:28:27.232 --> 00:28:29.344 that this is the region of the
nucleus where the DNA is exposed
00:28:31.192 --> 00:28:33.298 to the cytoplasm and where we’re
getting C gas recruitment.
00:28:33.298 --> 00:28:34.702 And so we would speculate from this
that this is the region of the
00:28:31.132 nucleus where the DNA is exposed
00:28:33.298 --> 00:28:34.702 getting C gas recruitment.
00:28:34.710 --> 00:28:36.980 And so this is just showing you here what
happens when we treat with PARP inhibitor.
00:28:38.880 --> 00:28:39.924 Sorry, I’ve lost.
00:28:41.602 --> 00:28:44.140 So the on the on the left is just
the UWB one, this UW one cell line.
And then when we add elaporib, interestingly one of the things that we see is the elaporib increases the percent of cells that have these persistent DNA bridges. But UWB ONE cells have abundant micronuclei as many tumor cells do in vitro. And actually, this is not precipitated by PARP inhibitors, at least in this context. And so this is another reason why we’re very interested in these bridges, because they seem to be the structure that’s most precipitated by PARP inhibitors, whereas there’s just a high rate of micronuclei all of the time.
But that does not seem to respond to the addition of, in this case, a lab rib. So we also think that for the vast majority of these persistent bridges that we observe in response to PARP inhibitors that there has been a loss of nuclear integrity. And so one thing I just want to point out here is that you know, one challenge I think in general is that you cannot see that that these nuclei have a persistent DNA bridge. If you just look at DNA stain because...
00:29:48.538 --> 00:29:50.547 it’s too thin essentially or there’s something about the DNA structure that disrupts the ability of the DNA stain to intercalate into the bases. One or the other, we don’t actually know yet. So actually in order to know that there’s a bridge there, you need a marker for a bridge. And actually it turns out that one of the best markers for a bridge is this protein called man one, which is a specific nuclear envelope protein. And so you know, you can see quite a beautifully that it is, you know,
In the nuclear envelope of all cells, but it really nicely decorates these bridges. And so this has been a really important tool. It seems very simple, but the ability to see the things that you want to look for is, is pretty important. So we’ve been using this antibody to this inner nuclear membrane protein, in order to surveil this. And so we can then look at the coincidence of other factors on these bridges and I want to focus specifically on the other elements of that DNA repair pathway.
So not only is C gas recruited and yet we interpret that as ruptured bridges, but there's also the recruitment of LEM two and bath. These are factors that are involved in trying to repair these breaks in the nuclear envelope and so this is evidence that the same kind of antagonism that I showed you in an induced rupture of the nucleus is also going on here. If we identify bridges using this 1 antibody what we can see is that all bridges have limb 2 which we expect.
membrane proteins but more than
half of them have C gas recruitment
and so this suggests again that
the majority of the bridges that we
detect are ruptured and that DNA
is likely exposed to the cytoplasm.
I also want to point out that
one of the ideas in that nuclear
envelope reformation is that there’s
local recruitment of LEM two and
these escort proteins to try to to
actually seal the nuclear envelope.
And if we kind of zoom in
particularly on LEM two, LEM 2 here,
there’s a really high accumulation of LEM 2.

And so this is likely the region of this bridge where there’s been a loss of integrity.

And that kind of explains why sea gas is also seen in this patchy pattern because there probably is a local effect.

And I’m just showing you here line profiles, just showing that there’s specific recruitment of LEM two, this man one protein, even though it’s in a nuclear membrane protein, it’s kind of distributed throughout the bridge and that it’s not part of the same repair network as LEM twos.
This makes sense to us.

I also want to point out that while though that work is in UWB ones, UDO in the lab has also been looking at a model of BRCA 1 deficient triple negative breast cancer. And so these again are cells treated with a laparib. This is just showing you the Dappy staining. I just want to this I think is a beautiful example of pointing out that even when we can’t really perceive these bridges in the DNA stain, these ones are a little bit earlier. So you can still kind of see.
faintly that there’s DNA staining.
You can appreciate the changes in nuclear shape that are tied to this, just like those heart-shaped nuclei in that first movie that I showed you with the nuclear localization signal. So there’s actually 2 hallmarks we think that we can use as essentially I know proxies for the presence of these bridges. One of them is the kind of orientation of these two nuclei. But the other is that there are these classic changes in nuclear shape that we see that are coincident with this.
we think about whether we can use the prevalence of these structures as a biomarker, which is one of our kind of long term interests. This is just showing you that in this MDA 436 line, preliminarily what we see is that there’s a dose dependent increase in the number of cells with these bridges in response to a laparib, whereas we don’t see this in a different triple negative line that’s BRCA 1 proficient and HR proficient. So I’ve shown you this that we likely
have these persistent bridges,

NOTE Confidence: 0.5707516

they accumulate in the context of a laparib,

NOTE Confidence: 0.5707516

they recruit C gas.

NOTE Confidence: 0.5707516

But is there actually activation

NOTE Confidence: 0.5707516

of the innate immune pathway?

NOTE Confidence: 0.5707516

Just to remind you that the canonical

NOTE Confidence: 0.5707516

pathway is that C gas produces

NOTE Confidence: 0.5707516

C gamp which activates sting

NOTE Confidence: 0.5707516

which phosphorylates TDK one and

NOTE Confidence: 0.5707516

IRF 3 and leads to interferon

NOTE Confidence: 0.5707516

stimulated gene expression.

NOTE Confidence: 0.5707516

So if we look at the GWB one cells

NOTE Confidence: 0.5707516

in the presence of a lab rib

NOTE Confidence: 0.5707516

compared to the vehicle control,

NOTE Confidence: 0.5707516

we don’t actually see the level

NOTE Confidence: 0.5707516

of TBK one phosphorylation,
But if we look at IRF 3 phosphorylation, we see that there is a stimulation of the phosphorylation of IRF 3. And if we look at the downstream consequence, which is interferon stimulated gene expression, just picking two of those genes, we do see that we can stimulate. We can see stimulation of interferon stimulated genes with the addition of ELABORA in this cell line. And just to point out this, how much of a signal we get does depend on how intact the C gasting pathway is. And many tumors have an activated...
C gas expression likely because there is selection against the pathway I’m talking about. But these cells do as you can see here, they do express sea gas and sting. But this is about as much stimulation as we can probably get in this line because this is an experiment where we’ve just transfected DNA to drive an innate immune response. This is the two people using this in this field all the time. And we get a pretty similar degree of stimulation as we get with the elaborate treatment. So that may be kind of the top of what
we can get in this particular cell line.

So we do think although this is only about four fold increase longitude full change of two that this is that this is a pretty strong response for this cell type.

So does the you know does this the response actually require C gas that stimulation of this innate immune pathway.

So now we’re just doing an experiment where we’re knocking down C gas and you can see the knock down by qPCR to the C gas gene. I’ll just walk you through this.
This is the same stimulation that we saw of these two genes with the addition of a lab rib. If we now knock down C gas, what we can see is that this does to some extent limit the activation. But to what extent that is we’re not, we’re not where I would say where it’s unclear yet whether C gas is completely responsible. We’re trying to be kind of very agnostic about what is lying downstream. And so one of the things we’re doing is generating C gas knockout Isagenix of these cell lines to really look at how much sea gas
is important for this

response and also of course

for the cell death mechanism.

One of the ideas that I set up was that

this nuclear envelope repair network

could be antagonizing surveillance by

the innate immune system and we have

some evidence that that’s the case.

So just to remind you,

the idea is that Bath and this

LEM two protein come in to recruit

escorts to seal these breaks in the

nuclear envelope and this limits

sea gas access and activation.

So here is an experiment where
we have used siRNA to knock down the bath protein to test this. So again here you can see the interferon stimulated gene expression in a lab with elaborative treatment. This is again in the UWB 1289 cells. Interestingly, and consistent with another study in the literature, if you just knock down Bath, you also get a stimulation of an immune signaling, which suggests that just knocking down bath and removing it can always stimulate some sea gas. And that may be as cells are reforming
their nuclear envelope or some other aspect of the normal cell Physiology. However, if we now add a lab rib, we can boost this even further, suggesting that there’s a synergistic effect of knocking down Bath and adding a lab rib, which suggests that a lab rib actually precipitates these kind of breaks in the nuclear envelope because of these entangled chromosomes. And then normally Bath would be suppressing the signaling downstream of that event.
But when it’s not there, we get more C gas expression. So this is consistent with that kind of antagonism.

So I just want to show you just briefly because it’s just you know we’re cell biologists so we love to look at things. This is this really cool reconstruction of what one of these bridges looks like up close. And I bring it up because the protein man one which is in yellow is actually localized to the mid body and the protein LEM 2 which is that nuclear repair protein you can see along this bridge.
actually in distinct regions. As I mentioned LEM two is likely to be along the regions of the bridge that are ruptured and actually man one is sitting on the mid body. And so one other area that that we’re interested in looking into is there is a known checkpoint that controls whether cells do abscission. That’s does seems to be downstream of surveilling whether there’s been chromosome entanglements and this is regulated by Aurora B, which is interesting because the Aurora kinases have also
been interesting clinically, although I think I’ve not been so far really terribly successful clinically. But I think that this is one context where thinking about how Aurora B might impact these events and be involved would be very interesting. So that and that is a reason why you get these doublet cells that are binucleate is because there has been an obscision failure downstream of the failure to segregate chromosomes. And so that’s something that we’re interested in in pursuing. So I just wanted to come back to this
idea that these nuclear integrity defects are the OR and these mitotic errors and then nuclear integrity defects.

Could this, could this be something that we actually take advantage of as a biomarker?

This is something that we're really is very preliminary, but we're very interested in. So you know as I've already pointed out, when you have these persistent DNA bridges, there is this relationship between the two nuclei, the result from that mitosis and there are these changes in nuclear shape.
These are actually H&E from the 10020 trial headed by Pat Larusso and as well as Kurt Shopper. And one of the things we’ve been looking at is if we look at these tumors in patients that are bracket deficient treated with a laparib, can we see these structures. And I think what we’ve been, we did not expect to be able to see any of these structures in H&E just to be honest. But but we’re kind of excited that we can see these kind of arrangements that are between cells.
they were not the first people to ever comment on this, but I think we’re connecting these observations to an underlying mechanism that may highlight why we should be paying more attention to the prevalence of these structures. I think particularly because micronuclei cannot be perceived in H&E, this may be a mitotic error that’s much more easy to perceive in the tissue and so might this. I think and one really interesting part of this to me is that you know there’s already an...
increase in these bridges just in HR defective cells that you can push further with PARP inhibitors. But this could be a kind of non genomic way of assessing is there a homologous or combination or just DNA repair defect in this cell line Because I see these mitotic errors that actually are such, so large that they can be perceived even in HNA. To really validate that we have to be able to actually, you know, convince ourselves that these
really are the structures that I've been talking about that we see in tissue culture cells. And so to be able to do that, we are working on validating some of the antibodies that we’ve raised to these specific nuclear envelope proteins. I mentioned it’s really hard to see these bridges even with DNA stain. You really have to have the right molecule that you’re looking for and we think that these integral and a nuclear membrane proteins are exactly that. And so we’re hoping to validate these structures indeed are representative of these DNA...
00:41:30.996 --> 00:41:33.146 bridges because we can specifically identify them with these antibodies. And then in addition, I think just to be a bit agnostic also, but other mitotic errors like Micronuclei LEM two in addition to being recruited to the ruptured regions of DNA breaks is also recruited strongly to ruptured Micronuclei. And so if we had this molecular tool, we might also be able to more accurately quantitate the prevalence of micronuclei and chemical samples, which would be fantastic. And you know why I think that’s so
important and I just picked out one example, I could have picked out many of them is that there of course is an interest in expanding PARP inhibitors beyond you know breast and ovarian, right. So I just pick and picked out one of these examples of the fact that there really are some amazing clinical responders. This is in pancreatic cancer here. There has been selection for BRACA associated pancreatic cancer, but I think anecdotally, we know there are triple negative breast cancers that respond to PARP inhibitors.
even if we don’t understand why.

There are right very aggressive prostate cancers,

A subset of which respond to PARP inhibitors even though we don’t understand why.

And so we’re hoping that these kind of biomarkers could potentially indicate where PARP inhibitors might be effective even when the molecular or genetic signature isn’t understood.

So just to just to restate what I’ve told you today,

while Laparov enhances the prevalence of these persistent DNA bridges,

there’s already more in
an HR deficient context.

But you can push this further with PARP inhibitors and this does lead to activation of innate immune signaling.

Their recruitment of bath and C gas may be antagonistic, but both are seem to be recruited to these bridges.

So that suggests that there many of them are ruptured. We're interested in whether just regulating disrupting this nuclear envelope repair network could actually further stimulate the innate immune signaling downstream of these mitotic errors.
And we’re excited about the idea of these persistent bridges could be an accessible hallmark of HR deficiency, which as I said, we poorly need in terms of what our next steps are and what we’re focusing on at the moment, where we really need to understand if this is really the canonical ISG expression is relevant here or perhaps there’s some other downstream consequence that’s running in parallel with the production of Isgs. That’s important. Again,
You get cell killing in a tumor cell intrinsic way in a dish. So we don’t know if that’s really a consequence directly of anything to do with ISG expression. And so that’s something that we’re exploring. We’re also taking both candidate approaches and unbiased screens to identify what are the factors required for the cell death in culture. You in some ways you would have thought this would have come out of CRISPR screens which have been done. But actually I think there are a lot of reasons to think that those screens weren’t really set.
up to identify this mechanism. And so that’s one of the things that we’re setting up to do at the moment. Again, we’re cell biologists, so we’re using correlative light and electron microscopy to really understand what’s happening in these DNA bridges and also to get information about the DNA structure. We can do that by looking at accessibility to the TN 5 transpose ACE as an example, which is the basis for ATAC experiments, but you can use that in a microscopy based experiment as well. And then we’re working with our
partners at AstraZeneca to really try to test whether we can use these bridges as a biomarker, you know, at the very initial stages in a really well controlled system. So one of the things that they have is that they have xenograft models of BRCA 1 deficient tumors which they then treated those mice with a laparib. And so we have really nice kind of ground truth data of HR deficient, HR proficient, you know, with and without treatment with a laparib or other PARP inhibitors. And so looking at the H and AE of
those data sets and doing that in a blinded way will really help us to understand whether this is something that's going to be worth pursuing.

All right. So I just like to thank the people who did the work and then I'm happy to take any questions.

We have a really great group working on genome integrity in the lab. Yuduo is a fellow much of what much work from the lab. Carrie recently joined the team and she's going to be working on these screens for DNA repair.
So we’re almost getting sorry not DNA repair screens to identify the mechanisms of cell death downstream of PARP inhibitors in the cell models. And I’ll say just joined the lab and he’s going to try to get our our tissue part of this up and going. I’d also like to acknowledge Pat Larusso who has really been essential and in all aspects of getting us involved in this direction. It would not have happened without her and I’m happy to take any questions. Thanks. Yeah. Have you seen this type outside of other HRD.
such as what do you thinking
Yeah I yeah I think we have not some
of the data that I showed you from the literature is strongly
suggestive that also in the contents of bracket two we need mitosis to get cell death you get innate immune signaling.
We have not, I should ask Connor actually but I don’t,
I don’t even think Connor we haven’t stained bracket 2 deficient cells.
So I don’t think we’ve explicitly done that just cause we’ve we’ve been focused more on BRCA one in our lab.
But I would be highly surprised if
it wasn’t the same in a probably probably
And and just to make the point you
know others have also seen similar
downstream effects for example of Taxol
treatment and actually have shown that
you know tumor cells that respond
to Taxol have intact C gas stings
signal and those that don’t do not.
So that I think that if this is not going
the same limited just to HR deficiency,
it’s just one of the ways that honestly
TAXOL HR deficiencies of HARP inhibitors
and and even a radiation probably could
all be stimulating the same pathway.
Yeah, as a fault.
So I mean if you're having an inhibition of the main signaling, would these cancers be potentially more sensitive to alcoholic viruses or kind of a you know alternative fall strategy? I think that’s a great question. And I think that as you can see what we’ve done, we’ve completely ignored right, any of that, any of that crosstalk. And I and I think it’s if you look in the literature it’s been kind of challenging and people who’ve tried to use this even even not even to the depth of what you just asked.
But if you look at you know is sting actually is sting signaling actually a tumor suppressive or a tumor driving mechanism, right, Because inflammation driven by sting has also been suggested to be a driver, right. Is, is actually a tumor driver C gas I think. And actually if you look at the number of tumors that have inactivated C gas versus sting, very few inactivate sting, the vast majority have inactivated C gas if you just look across you know that map. And so I do wonder if some of the signaling we’re seeing is C gas dependent,
but maybe not strictly through sting or sting is more complicated because it’s multiple roles and I think that might be important to tease out to think about. Then how is this going to intersect with approaches like oncolytic viruses. So I think that’s still one of the confusions at the moment because honestly there’s very high profile papers saying you know sting agonists would be great and sting agonists are terrible. And so that’s probably going to be context dependent.
Go ahead. I'll get the Mario and I'll get this.

In the meantime, are there about cell lines that are HID deficient where you could look at a lab rib in one of these cell lines and in the meantime determine if the immune activation is important or not. I really agree with you. So I think that is an excellent experiment.

It is an experiment that needs
to be done and it you’re right, it’s it’s obvious and it’s achievable. It hasn’t been what our expertise has been in, but I agree with you that that’s exactly the right. So we really need some genetic models to be able to, to do that. So I I completely agree Jeff has asked wholexome sequencing is not as commonly performed as H&E, but he’s curious which degree of mutational signature derived from wholexome sequencing indicates the effective homologous recombination.
or is being used as a biomarker.

So to Jeff's point, yes, this is the only biomarker there is,
is a kind of scoring genomic scarring.

But the challenge I would say is, and I think Jeff will appreciate this,
is that the cell may be HR defective now,
but then it may become a resistant to PARP inhibitors because it’s
now HR proficient and it will still
have the scarring left from the period where it was HR deficient.
So that may help us to understand
you know context.

We don’t have any reason to think
someone has a germ on or somatic
mutation that they could benefit for a PARP inhibitor because we see that. But I’m not sure we’re looking for that signature when there’s no reason to be from the genomics already. So I don’t think we’re doing that. We absolutely are using that when there’s a reason to think that there is A and HR defect, but it can’t tell us. It only tells us the history, it doesn’t tell us presently what’s happening in the tumor.
And so I think that’s the limitation.

Thanks for your question.

With the bridges, are those all contained in cytoplasm or do those potentially contend kind of extra targets for antibodies and cars or something like that that would be unique to I think it’s a great question whether you ever I think that there’s I don’t think we ever see that the plasma membrane right actually ruptures although you know escorts also repair holes, temporary holes in the plasma membrane. So I won’t say that we’ve actually tested that and that would be really interesting to know whether that’s the case.
I mean it’s interesting that these same factors actually man one in particular were all identified as being auto antibody. They are all tied to auto antibody to autoimmune diseases as common targets of many nuclear proteins are. But I do think that that’s interesting and there’s some evidence that that the Lam 2 protein also probably in the absence of functional M2, you do have kind of a prevalence of autoimmunity which would be consistent with not being able to do this normal cycle of Nuvo Con number reformation does get surveilled through this.
00:52:51.830 --> 00:52:53.758 mechanism and and can be deleterious.
NOTE Confidence: 0.8885353
00:52:53.760 --> 00:52:55.356 So I think it’s but yeah,
NOTE Confidence: 0.8885353
00:52:55.360 --> 00:52:57.208 we we don’t really what we see is
NOTE Confidence: 0.8885353
00:52:57.208 --> 00:52:58.946 that you know likely eventually most
NOTE Confidence: 0.8885353
00:52:58.946 --> 00:53:01.312 of those cells will give up and I
NOTE Confidence: 0.8885353
00:53:01.312 --> 00:53:03.209 think it’s just a just a highlight.
NOTE Confidence: 0.8885353
00:53:03.210 --> 00:53:05.847 This is why one has to be careful in
NOTE Confidence: 0.8885353
00:53:05.847 --> 00:53:08.101 assessing in this area particularly facts,
NOTE Confidence: 0.8885353
00:53:08.101 --> 00:53:09.847 profiles looking at cells that look
NOTE Confidence: 0.8885353
00:53:09.847 --> 00:53:11.543 like they’re G2M cells because you
NOTE Confidence: 0.8885353
00:53:11.543 --> 00:53:13.335 get these cells that are G2M cells
NOTE Confidence: 0.8885353
00:53:13.394 --> 00:53:15.361 but they’re actually in G1 and that’s
NOTE Confidence: 0.8885353
00:53:15.361 --> 00:53:16.994 because they failed in cytokinesis.
NOTE Confidence: 0.8885353
00:53:16.994 --> 00:53:19.738 So now they’re 4 N but they’re
NOTE Confidence: 0.8885353
NOTE Confidence: 0.8885353
00:53:21.490 --> 00:53:23.002 And so that’s one of the
NOTE Confidence: 0.8885353

00:53:23.002 --> 00:53:24.370 things that we see here.

NOTE Confidence: 0.8885353

00:53:24.370 --> 00:53:25.768 So it'll show up in experiments

NOTE Confidence: 0.8047927

00:53:33.110 --> 00:53:35.000 all right.