The Cancer Center grand rounds.
I’m Barbara Burtness, and I’m really extraordinarily thrilled to be introducing smuggler Jessica as our speaker today. She is an associate professor of genome maintenance at the Rockefeller University. She received her PhD from Rockefeller and her MD from Wild Cornell Medical College. Following a residency in clinical pathology at MGH, she joined Harvard Medical School as a postdoctoral fellow in Stephen Elledge’s lab.
She's the recipient of numerous awards, including the Irma T Herschel Research Award, the Rita Allen Foundation Scholars Program Grant, the Burroughs Welcome Fund Career Award, the AV Foundation Translational Research Award, and the Herald Weintraub graduate student. Her lab focuses on DNA repair mechanisms, particularly those involved with interstrand crosslink repair pathways, and she's interested in identifying new genes that are involved in this repair process in order to reveal the mechanisms at play in human diseases that result from deficiencies in interest strand cross link repair.
This includes Fanconi anemia and kerio megalith interstitial nephritis. To this end, she successfully identified SLX 4, red 51 and U BE2T is interstrand crosslink repair factors, as well as developed a mouse model of Carrie Magalog interstitial nephritis. And recently she’s begun studies revolving around how replication stress is addressed by cells to promote genome stability. She also has a deep interest in those cancers which arise in patients who have DNA repair.
Defects.
And it’s because of this that I’ve had the extraordinary pleasure of beginning to work with her on the stand up to cancer Grant, which looks at Fanconi anemia, associated head neck cancer. So. Like I said, if you wanna come up. This plaque is to thank you for coming to give grand rounds in person, coming to give grand rounds in person, which many people have not been willing to do recently. And we’re really looking forward to your talk.
Thank you so much. Yes, thank you.

Well, thank you Barbara for first of all for inviting me all of you for coming to hear the lecture and all of you on zoom for joining in.

And it’s really my pleasure to to be here and it’s been fantastic to work with Barbara and and learning from her more about the the clinical aspects of what we do and hopefully bringing some new therapies to these patients eventually, OK, so.

I will talk about DNA interstrand crosslinks I'll just remind you though that oh,

these are my disclosures.
I’ll just remind you that DNA repair is really essential for a lot of aspects of life and depending on what kind of flavor of DNA damage you have, whether it’s from the outside, from the UV light creating these dimers, or from within the cell, something that I’m quite interested in, for example, creating mismatches. There is a repair pathway that’s dedicated to these and there are a number of genetic diseases associated with inability to repair these different lesions and they come again in many different phenotypes resulting in developmental abnormalities and the generate the
generative diseases and a lot of them obviously are associated with cancer prone due to mutagenic nature of these lesions if the lesions are not properly repaired. So my favorite lesion is this interest in Cross link and I'll give you a little bit of introduction about these lesions. This is a covalent linkage of two strands of the DNA. It’s repaired by the Franco anemia DNA repair pathway and I’ll give you more of a of the mechanism of how this is done in a bit in later slide, but the whole purpose of this pathway...
which is activated during DNA replication.

Is to create 2 pristine.

Double stranded DNA that can be then moved to dollars trends during during the cell division and I'll just mention I won't talk much about it but this pathway is also activated at difficult to replicate regions.

So repetitive DNA are loops and there is growing evidence that at any time there is stalling of replication forks the pathway can come to rescue. Those forks so that this this intern crossing is really our model of how pathways activated. And the outcomes of abnormality in
pathway activation or function are these genomic instability that you can heat see here in this metaphase spread where you see number of these abnormal radial chromosomes. So we see that in cells from Franconia anemia patients when they are treated with external internal crosslink causing. Chemicals like mydomain sincere splatsin you see number of abnormalities including gaps, breaks and these radio chromosomes occur because of two double strand breaks that are that find each other and are joined together forming these radios.
And obviously these radios come radio chromosomes can cause instability but can also cause death of cells when they are trying to divide. So the reason why we know that this pathway is particularly important in stem cells are all these phenotypes that we see in patients with Fanconi anemia. There are many developmental phenotypes including these skeletal abnormalities that you see here, but pretty much any system can be affected and that’s seen in patients who are truly null for this pathway. Majority of patients will present with Pance, Cytopenia.
Around age, median age of 10, and even though it’s called anemia, all of the hematopoiesis can be affected and actually platelets are usually the first to draw. And then there is very interesting phenotype that will spend a lot of time talking about the cancer predisposition MSDS and AML occurs in the setting of pancytopenia and squamous cell carcinoma occurs later in life but still in at young age of average 31. We have very young patients as I’ll show you in future.
slides and then depending on the actual path of gene that’s mutated. We can also have breast cancer, medulloblastoma and other embryonal tumors. And there are other phenotypes that are still fairly poorly understood like infertility and endocrine abnormalities. So this is a great model in my mind for studying DNA repair. So today, I’ll tell you about the first part, very short part about the mechanism of interstrand crosslink repair. Secondly, I’ll talk about the identification of endogenous sources,
00:07:56.670 --> 00:07:58.826 sources of interstrand crosslink.

00:07:58.826 --> 00:08:02.060 And today I'll talk about the bone marrow side.

00:08:02.153 --> 00:08:03.608 But we are very much interested in those in the keratinocytes as well.

00:08:03.610 --> 00:08:05.668 And this is an ongoing work in my lab.

00:08:05.668 --> 00:08:08.000 And then I'll talk about the mechanism of tumorigenesis and Fanconi anemia.

00:08:08.000 --> 00:08:11.186 Especially in the squamous cell carcinoma.

00:08:11.190 --> 00:08:16.280 So just to give you a flavor of how this pathway actually functions,

00:08:16.280 --> 00:08:19.430 this is a very regulated process of repair of these lesions.

00:08:19.430 --> 00:08:23.390 So we start with the ACL that you have shown you before and this...
is the structure that we know forms when the replication forks stall at the lesion and some of this work has been done in human cells, some of it. This work was done in Johannes Walters lab in the Xenopus egg extract system. So biochemically, it's a fairly understood pathway. What's essential in this pathway, and I'll show you the proteins that are involved in a moment, but what has to happen is that the DNA needs to be broken, in a very regulated way that then gets repaired through translesion.
synthesis and then through homologous recombination that requires BRC 2 and other proteins. And this eventually leads to this full DNA repaired DNA with two strands that are fully repaired. So the proteins that are participating in this pathway really has been identified through studies of Franco anemia patients and. Just go to go back for a moment to the history of this disease. Fanconi anemia has been identified in 1927 by Guido Fanconi and described as hereditary pancytopenia with.
short stature and hyperpigmentation.

The first gene fancy was identified 92,

but even then it was known that more

than one gene will be causative

And this was Buckward lab who

identified the first gene.

And now we know that there are 22 genes

that can be mutated in Fanconi anemia

and we actually are working on on #23,

which fits very nicely in this pathway.

And the this data is actually showing

the percentage of of patients with

different mutations in different genes

and this is from our International

Franconia registry that I,
I run at the Rockefeller University. And you can see that Frank a mutations franc and Frank G mutations are the most common. More majority of the diseases of this disease is autosomal recessive we have. Language which is excellent and then uh thank our that we’ve discovered right 51 mutations. These are always the Novo dominant and we have a number of patients now with different mutations and and rat 51 and all of them have the same dominant function and they’re all presumably because right 51 is necessary during biosis and we
would never recover any any patients,

any transmission really through

through the germline.

OK.

And this registry that I've mentioned

has been really instrumental for

our work and it was started in

1982 by Arlene Auerbach who's still

participates in the lab meetings

and has a lot to say about about

the disease and has been really

wonderful in in helping us through

this through some of these studies.

So now I'll spend really few just few

minutes on the proteins themselves.

Just to give you a gift,
again a flavor for the for this pathway. So majority of the proteins whose genes are mutated in Fanconi patients form this large core complex. All of the colored proteins are actually those that have mutations in Fanconi patients. And you can see that this core complex is situated that this cross link as if it was identifying the the damage. It’s actually unclear. How that’s exactly done?

The key to this core complex is that it has this E3 ubiquitin ligase fancl and with Frank T, which is the E2.
they the whole purpose of this core

we we understand now that the whole

purpose is to ubiquitinated Frankie

and Frankie 2 and that process

through beautiful structural studies

Semoran Pablo Ditches lab

Who forms a clamp around the the crosslink?

Actually there are more,

probably more clamps around as

shown by the Dean slab that sort

of decorate this cross link.

Eventually,

and this is again not quite worked out,

the proteins that are important for
NOTE Confidence: 0.844819513636364
00:13:19.982 --> 00:13:22.794 in making these incisions come in
NOTE Confidence: 0.844819513636364
00:13:22.794 --> 00:13:25.140 and one of the important proteins
NOTE Confidence: 0.844819513636364
00:13:25.140 --> 00:13:28.519 here is this SLX 4 which acts as a
NOTE Confidence: 0.844819513636364
00:13:28.519 --> 00:13:30.694 scaffold for three different nucleases.
NOTE Confidence: 0.844819513636364
00:13:30.694 --> 00:13:34.523 And it's quite amazing how the three
NOTE Confidence: 0.844819513636364
00:13:34.523 --> 00:13:36.760 nucleases are associated with one
NOTE Confidence: 0.844819513636364
00:13:36.760 --> 00:13:39.820 protein and frank P is or a cell.
NOTE Confidence: 0.844819513636364
00:13:39.820 --> 00:13:43.320 X4 is essential not only for scaffolding.
NOTE Confidence: 0.844819513636364
00:13:43.320 --> 00:13:46.336 But for the function of the of these
NOTE Confidence: 0.844819513636364
00:13:46.336 --> 00:13:48.276 other nucleases without this slex
NOTE Confidence: 0.844819513636364
00:13:48.276 --> 00:13:50.866 4 none of the nucleus is actually
NOTE Confidence: 0.844819513636364
00:13:50.940 --> 00:13:53.190 are functional within the cell.
NOTE Confidence: 0.844819513636364
00:13:53.190 --> 00:13:54.882 They have full in vitro function
NOTE Confidence: 0.844819513636364
00:13:54.882 --> 00:13:56.550 but not not cellular function.
NOTE Confidence: 0.844819513636364
00:13:56.550 --> 00:13:59.370 So it’s it’s fascinating but in
NOTE Confidence: 0.844819513636364
this case XPF is the one that’s making the incisions.

I’ll also mention the protein that Barbara mentioned the carrier Magali Constitution nephritis which has mutations.

In fan one and Fan 1 gene codes for this another nuclease and this system which can also unhook, but it can unhook this this cross link outside of S phase and we’re still very much interested in knowing how that affects the function especially in the kidney and and the liver.

Eventually, after these, uh,
these incisions are made,
you bring in translesion polymerases and they are used to replicate
across this unhooked lesion.
And that’s actually probably not a mutagenic process. It’s mostly a Posada that can repair this lesion.
And eventually, as I mentioned, there’s a whole homologous combination pathway that is involved in a repair of the double strand break and here all of your proteins, favorite proteins that are associated with breast and ovarian
cancer when they’re mutated when

the genus mutated in one copy.

So BRC 2, power B2B,

RC1,

rad 51 are functioning in this pathway and eventually we go back to SLX.

Or and associated Mercedes one and

the selects one where the repair needs to be completed using these nucleases or a bloom healer case.

I will also mention that there is another pathway that’s and

the reason why the RCA two and Route are necessary at these cross links and that’s to protect these cross links from nuclease and
Our lab has shown that there is a protection against DNA damage. Warner and other labs before us have shown for especially the Jason lab that there is also protection against MRE 11 at stalled forks for example, after hydroxyurea and other damage. So this is all I have to say about the mechanism of function of these proteins. There are still a lot of details that need to be filled in, but we have an idea of how this pathway functions.
And the only other thing that I say is that it’s really a very well regulated process, so you make double strand breaks. But they are immediately being shuttled to the proper repair. OK, so we’ll talk about what happens in the third part of my talk, when this repair is abnormal and the brakes are still made, but they are inappropriately repaired. So in the second part, I’ll talk about the endogenous sources of interest and cross links.

So I showed you that the Franconia pathway
00:17:22.840 --> 00:17:25.508 is necessary for repair of I CL’s,

00:17:25.508 --> 00:17:27.275 but you may ask, well,

00:17:27.275 --> 00:17:30.485 where are these IC’s coming from?

00:17:30.490 --> 00:17:32.255 Obviously in the in the

00:17:32.255 --> 00:17:34.020 clinic we are very much.

00:17:36.700 --> 00:17:40.137 Aware of cisplatin and mitomycin C and

00:17:40.137 --> 00:17:42.686 interest rate crosslinks occur after

00:17:42.686 --> 00:17:45.668 treatment with these with these agents,

00:17:45.670 --> 00:17:46.830 although there are other

00:17:46.830 --> 00:17:48.280 lesions that occur as well,

00:17:48.280 --> 00:17:51.440 right intrasrand crosslinks and others.

00:17:51.440 --> 00:17:54.576 The exogenous damage also can come from

00:17:54.576 --> 00:17:58.018 bacterial metabolites, and there are.

00:17:58.020 --> 00:18:02.045 There are E coli strains that are

00:18:02.045 --> 00:18:04.191 making ICL inducing. Chemicals.

27
It can come from acetyl, aldehyde, from ethanol.

It can come from tobacco smoke, which is full of formaldehyde, acrolein and other mutagens.

But formaldehyde and acrolein are very good into strong cross linkers.

But we are particularly interested in sources of endogenous DNA damage and really beautiful work that I’ll describe a little bit in the two slides that are coming up have shown that endogenous toxic metabolites formaldehyde and acetaldehyde are particularly important in the bone marrow and definitely contribute to bone
marrow failure in Frankonia patients. And in mouse models, so let me just introduce them. This work was done mostly by KJ Patel’s group in the UK and they’ve defined LDH 2 and ADH 5. These are alcohol and aldehyde dehydrogenases as being important for this first tier of protection. So they remove the toxins from the cell and that prevents DNA damage and of course the Frankonia pathway is a second tier of protection. Whatever has been incorporated as
cross links will be removed as well.

So let me just give you a segue and talk about a LDH 2 and ADH 5. So a LDH 2 is necessary for removal of acetyl aldehyde which can come from ethanol from the outside, but also from metabolism. And LDH 2 is responsible for detoxifying it to acetate.

And a KG Patels group has described LDH 2 knockouts when combined with F angy 2 knockouts. The mouse for the first time really developed bone marrow failure and leukemia.
So the mouse model without just fanced
2 mouse model does not develop.
Bank bone marrow failure or leukemia.
It has mild hematopoietic dysfunction,
it’s nothing that you consider
a bone marrow failure.
In humans combination of FANK
mutations and usually it’s frank,
a mutation and a LDH 2 star 2
which is the dysfunctional.
Variant of LDH 2 causes early onset
a really beautiful paper from
the Takata group that have that has
shown that and that was published
31
00:20:56.798 --> 00:20:59.348 in 2013 which really solidified
NOTE Confidence: 0.753543265
00:20:59.348 --> 00:21:02.929 our thinking about this this as a
NOTE Confidence: 0.753543265
00:21:02.929 --> 00:21:05.419 as a modifier of Fanconi anemia.
NOTE Confidence: 0.753543265
00:21:05.420 --> 00:21:08.535 And I also will mention that together
NOTE Confidence: 0.753543265
00:21:08.535 --> 00:21:11.469 with Chris Vakoc’s lab we showed that.
NOTE Confidence: 0.753543265
00:21:11.470 --> 00:21:15.610 Somatic Aldh 2 mutation silencing
NOTE Confidence: 0.753543265
00:21:15.610 --> 00:21:19.330 in AML’s results in in dependency
NOTE Confidence: 0.753543265
NOTE Confidence: 0.753543265
00:21:21.650 --> 00:21:24.618 So when when Chris’s lab and they
NOTE Confidence: 0.753543265
00:21:24.618 --> 00:21:27.875 are at Cold Spring Harbor did a
NOTE Confidence: 0.753543265
00:21:27.875 --> 00:21:30.701 screen and showed in number of
NOTE Confidence: 0.753543265
00:21:30.710 --> 00:21:33.870 AML’s dependency on Fanconi anemia.
NOTE Confidence: 0.753543265
00:21:33.870 --> 00:21:35.998 He called us up and and asked what
NOTE Confidence: 0.753543265
00:21:35.998 --> 00:21:38.277 what should I look at why are they dying?
NOTE Confidence: 0.753543265
00:21:38.280 --> 00:21:40.870 And I said well just look at LDH 2 I
NOTE Confidence: 0.7890958325
00:21:40.950 --> 00:21:42.965 bet it’s. Yeah, 2 and that’s what
NOTE Confidence: 0.7890958325
00:21:42.965 --> 00:21:44.729 that’s what it turns out to be.
NOTE Confidence: 0.7890958325
00:21:44.730 --> 00:21:47.178 We just helped him a little bit but
NOTE Confidence: 0.7890958325
00:21:47.178 --> 00:21:49.974 some of some of AML’s depend on on
NOTE Confidence: 0.7890958325
00:21:49.974 --> 00:21:52.380 Franconia because of the of the
NOTE Confidence: 0.7890958325
00:21:52.466 --> 00:21:56.214 silencing of LH2 which I think is quite
NOTE Confidence: 0.7890958325
00:21:56.214 --> 00:21:58.567 interesting and has implications for
NOTE Confidence: 0.7890958325
00:21:58.567 --> 00:22:01.458 for therapies for these for these AML.
NOTE Confidence: 0.7890958325
00:22:01.460 --> 00:22:04.920 So then ADH five came.
NOTE Confidence: 0.7890958325
00:22:04.920 --> 00:22:09.112 And that that was also from KJ Patel’s
NOTE Confidence: 0.7890958325
00:22:09.112 --> 00:22:13.117 work that ADH five is necessary for.
NOTE Confidence: 0.7890958325
00:22:13.120 --> 00:22:15.336 I mean this is this has been known
NOTE Confidence: 0.7890958325
00:22:15.336 --> 00:22:17.607 that has been known that ADH five
NOTE Confidence: 0.7890958325
00:22:17.607 --> 00:22:19.728 is necessary for detoxification of
NOTE Confidence: 0.7890958325
00:22:19.728 --> 00:22:22.548 formaldehyde through this through this
NOTE Confidence: 0.7890958325
00:22:22.548 --> 00:22:25.960 pathway and again they create a DH,
Five negative mice and they had bone marrow failure but also had glomerular damage. That's actually poorly understood still and there is this. New digenic human disease of LDH2 ADH5 double knockouts or the dysfunctional alleles which have bone marrow failure, myelodysplastic syndrome, foot for some reason, foot skeletal abnormalities and also neurodegeneration. So now you have phenotypes that are associated with ACL's and creation of ACL's in multiple different tissues.
Including in the brain and we if somebody is interested in knowing why the brain, we can discuss that later. But there that really shows us that there is a lot of. Well, the cells have a lot of different pathways for as this first tier of protection and my lab has been interested in identifying other pathways in different cells as cell types and we started with with cells of jurkat cells which are T cell leukemia cells to figure this out. So our hypothesis was that there
would be more of these detoxification path pathways in the cells and by understanding what that is. They might actually be equipped with understanding of what can we increase to have some preventive measures in in Fanconi anemia. So this is work from Munjung Jung who’s now a assistant professor at Hopkins and she was a clinical scholar in my lab and she did a metabolism focus screen. Performed in jurkat cells. And she took Frankie to positive and negative jurkat cells.
And the whole purpose was to identify the genes that are dropping out from fangy to negative cells. So these are essential, would be essential for faculty to cells. And you can see that there are lots of genes that that dropped out and some of them were actually consistent with what we know. So these are these cells, SLC 7A. and Assoc 3A2. They form a complex that is responsible for cysteine movement across the cell and that feeds into glutathione, which is important for.
formaldehyde detoxification,

so that fit.

There was also a we did get a

DH5 in the screen,

but the gene that we concentrate

on concentrated on was a LH9A1.

Eight out of 10 guides scored in

this in this assay and the rest

of the work was really based on a

LH9A1. This is a aldehyde dehydrogenases

in metabolizes amino aldehydes,

at least in vitro and it’s highly

expressed in liver, muscle and kidney.

And the idea here is that it will there

will be tax toxic metabolite that is

now detoxified by AL DH9A1 and this.
If you don’t have aldh 91, the toxic metabolite will create DNA damage and you don’t have if you don’t have Franconia pathway, these cells would die and get transformed. But if you have Franconia pathway, you would have cell survival. So umm munjung has validated this in multiple assays, and there is a bio archives paper that we’ve we put out there competition. Assays cells that didn’t have a and a LDH 9A1 did much more poorly and growth assays. She also could show that there were
increased numbers of apoptotic cells,
increased DNA damage through gamma H2AX.
And increased chromosome breakage.
So this is where we can look at
without exogenous DNA damage.
Look at numbers of breaks that are occurring in the double knockouts in different different clones and we can see that there is an increase of chromosome breakage if we don’t have a LDH 9A1 Infinity 2 cells.
She also used human hematopoietic stem cells depleted Frank A with SH.
RNA’s did a knockout with of LDH 9A1 and those could make fewer colonies.
But what happened?
We made a mouse expecting some level of bone marrow failure and we've seen phenotypes so these mice are born smaller than fanki. Negative mice which are already small have increased number of eye abnormalities at birth, which has been seen as a DNA. Damage outcome and they have increased number of variant tumors in aged mice, but really very mild hematopoietic defect. So that tells us that there will be differences also between mice and men in how the these detoxification pathways are working.
And this is why we when we are modeling things in the mouse with in this in this pathway I think it’s important to do things and in parallel in human system and in the mouse. And and compare and contrast because there will be differences and whatever we model in the mouse actually might not be as as important in humans and and vice versa. So the last question that we wanted to ask is, well, we’ve identified the the enzyme, but what’s really the, the problem, what’s the source, what’s the toxic metabolite that these
cells are dealing with so much junk

did a suppressor screen as we are,

we are geneticists at heart.

So she took the double negative cells

frankly to a LH9A1 double knockouts and.

I redid the screen the the same method

with using the same metabolism library,

but now wanted to see the cells

that are actually growing better.

So now we are knocking out some other

gene that is necessary for production

of our toxic metabolite.

And the Omni gene that we’ve

identified is this ATP 13A3.

We’ve actually identified a LDH 9A1 itself,
but that’s we think is actually through reversion mutations.
So these are now we are creating a mutant and a LDH 91 that reverts the function.
So we in a way it shows that our screen worked in both ways, but this gene is quite interesting because this is now.
A protein that’s necessary codes for a protein that’s necessary for polyamine, for example spermine.
Movement through the from between different membranes and if we have high level of spermine, we know at least this is what we think would happen.
We would get high levels of I mean the proper now and as I mentioned I’ll ADH 9A1 is involved in detoxification of amino propanol and propanol can lead to acrolein and cells. We actually haven’t yet shown that this these are this is what happens in the system. But everything that we have is consistent with this, with this hypothesis, so. If we have triple mutants, these cells, these cells now can grow much better, presumably because we are sequestering the polyamines outside of the away from the DNA, not creating these crosslinks. So I think this is an example.
00:30:53.491 -- 00:30:55.479 of how we are.

NOTE Confidence: 0.861485793333333

00:30:55.480 -- 00:30:58.070 Identifying endogenous types of DNA

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00:30:58.070 -- 00:31:01.204 damage that are necessary for crosslink

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00:31:01.204 -- 00:31:04.389 repair for that are necessary to be

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00:31:04.389 -- 00:31:07.114 repaired by franklinia pathway and

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00:31:07.114 -- 00:31:10.004 other pathways of crosslink repair.

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00:31:10.010 -- 00:31:14.734 And really adding to this idea of

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00:31:14.734 -- 00:31:19.170 how the first tier of protection is

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00:31:19.170 -- 00:31:22.020 necessary to to protect the the genome,

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00:31:22.020 -- 00:31:25.000 not creating interstrand crosslinks.

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00:31:25.000 -- 00:31:27.748 And that eventually obviously is necessary

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00:31:27.748 -- 00:31:30.770 for normal cell and organ function.

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00:31:30.770 -- 00:31:32.210 So with that,

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00:31:32.210 -- 00:31:35.090 I’ll move to the last topic,

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00:31:35.090 -- 00:31:37.808 which is cancer and Fanconi anemia,

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00:31:37.810 -- 00:31:42.190 children and young adults.
And when you think about anemia pathway, there are, I already mentioned that there are different types of tumors that can form and one of the tumors that we think about are these embryonal tumors and AML that are forming when homology directed repair is absent. So these are patients who have biallelic BRC 2. Or probably 2 mutations and they are identified early age you know they have these tumors within the first five years of age.
00:32:22.928 --> 00:32:25.949 their life and we actually have a
NOTE Confidence: 0.861485793333333
00:32:25.949 --> 00:32:28.254 mouse model of of medulloblastoma
NOTE Confidence: 0.861485793333333
00:32:28.254 --> 00:32:30.188 that is quite interesting but
NOTE Confidence: 0.861485793333333
00:32:30.188 --> 00:32:31.868 not ready for prime time.
NOTE Confidence: 0.861485793333333
00:32:31.870 --> 00:32:34.600 But the the reason why we are
NOTE Confidence: 0.861485793333333
00:32:34.600 --> 00:32:36.698 interested again in these tumors
NOTE Confidence: 0.861485793333333
00:32:36.698 --> 00:32:39.230 is because we want to understand
NOTE Confidence: 0.861485793333333
00:32:39.230 --> 00:32:41.670 why the granule progenitor.
NOTE Confidence: 0.861485793333333
00:32:41.670 --> 00:32:44.351 Cells or the cells that lead to
NOTE Confidence: 0.861485793333333
00:32:44.351 --> 00:32:46.060 neuroblastoma or tulips tumors,
NOTE Confidence: 0.861485793333333
00:32:46.060 --> 00:32:49.636 why do they really need BRC 2 function?
NOTE Confidence: 0.861485793333333
00:32:49.640 --> 00:32:52.004 What’s so special about these cells
NOTE Confidence: 0.861485793333333
00:32:52.004 --> 00:32:55.439 that require BRC 2 function or probably
NOTE Confidence: 0.861485793333333
00:32:55.439 --> 00:32:57.815 2 function homologous recombination.
NOTE Confidence: 0.861485793333333
00:32:57.820 --> 00:33:00.214 So that’s something that we are
NOTE Confidence: 0.861485793333333
00:33:00.214 --> 00:33:02.500 we are continuing to develop.
NOTE Confidence: 0.861485793333333
But outside of homologous recombination when the ICL repair is abnormal, so these are patients with mutations in any of the core complex fanki FANGY 2 SLX 4. We don’t have yet patients who have tumors with XPF, but that’s more of a problem of the PF being necessary also in other tissues. These patients develop AML’s and also develop squamous cell carcinomas and today we’ll concentrate on squamous cell carcinomas. So this is a knowledgeable audience about squamous cell carcinomas and we’ll concentrate on head and neck.
And in sporadic cases, you have two types of head and neck cancers. One of them is HPV associated that we want to spend too much time talking about. The other one is a HPV negative and those tumors are associated with carcinogens that are present in tobacco and alcohol. And I would also claim that endogenous aldehydes will play a role in here as well. And maybe for franconi patients who might not be exposed to alcohol and tobacco as much as the general population are, those indulgence aldehydes will be important.
The truth about head and neck cancer is that patients are diagnosed late, the survival is still poor and it’s not really decreasing. If it’s decreasing, it’s just because there’s more HPV positive cancers. The treatment can be quite horrendous for the patients and really learning from Barbara. The need is really to stratify these patients to also identify novel therapeutics that might not be DNA damaging. So, my lab got interested in these tumors because of our registry and tumors and Fanconi anemia patients just observing.
the patients who are becoming young adults,
especially bone after bone marrow transplant.
But even without the bone marrow transplant,
many of these patients have developed
cancer and in 2003 there was a paper
from the registry showing that patients with
Fanconi anemia had 700 fold increase of tumorigenesis in the
head and neck cancer in head and neck
area and for vulvar cancer and for cervical cancer and anal cancer there
were thousandfold increases in these.
So these are cancers that are squamous cell
carcinomas in Fanconi anemia patients.
And they are very there.
There are present in the tongue gingiva and buccal mucosa, some pharynx and larynx, but a lot of cancers in the oral cavity. We do have some esophageal cancers as well and those are quite interesting, but all of them have similar genetic or molecular characteristics. So here is our cohort of patients whose tumors were sequenced and you can see that the agent diagnosis is on a median of 31 years old.
but we have some 13 year old 16 year olds with head and neck cancer that’s extremely aggressive and extremely difficult to treat since we cannot use this platin for for these patients and they are radiation sensitive as well as patients although not that radiation can still be used carefully you can see that these patients don’t do well at all much worse than the. Sporadic cancers and patients who are here and survive long usually are patients who had successful surgical resection of their primary tumor. Or occasionally radiation therapy was successful.
OK, so we were very much interested in understanding the molecular pathogenesis in these tumors and we showed that majority of these tumors were HPV negative instead majority of them had P53 mutations and the P53 mutations were of variety, nonsense, missense, frame shifts and deletions. But P53 was pretty much the only Gina gene that was mutated through point mutations, All the other changes were actually associated with structural variants.
So here you can see that these are number of mutations in across different cancers through TCG data from TCG and I inserted our Fanconi tumors and they have point mutations, the number of point mutations. It’s pretty low and these tumors are lower than in sporadic head and neck cancers. And if we look at the what kind of signatures are present, there is no homology, directed repair or smoking signature and a lot of these are signatures of cell division. So the reason why we think that they have smaller, fewer. Mutations is because they are...
present in younger patients.

And instead what we see is this huge genomic instability where this is a circus plot and all of these, all of these.

Lines indicate translocations and structural variants that are present in these tumors and if we look at these tumors the there is about 2 to threefold increase when we compare it to HPV negative tumors and HPV positive tumors have very few of these since they already mutated P53 and RB and really don’t have to.
rely on these structural variants.

And this is comparable to the structural variants that we see in BRC 2 or BRC 1 tumors which have high levels of structural variation.

As far as types of structural variance, we see deletions, translocations, inversions, all of them are increased.

And if you take proportion of structural variance, actually the on the there is a slight increase of tandem duplication.

So pretty much across all structural variants there is an increase.

Which is consistent with breaks being...
made when the DNA Fanconi pathway cannot function and these breaks being repaired inappropriately and to show you the extent of how inappropriate this repair is we used number of techniques of long read sequencing and you can see that pieces of chromosomes are from different chromosomes chromosome 8. For 1511, they’re all sort of strung together in these tumors, creating these completely abnormal abnormal structures. So we also asked how these...
DNA molecules come together, whether there’s something special about them and it’s many of you know if you have a double strand break you, it can be repaired through three different mechanisms, non homologous end joining, microhomology mediated repair and single strand annealing. And all of these should be functional in Franconia negative cells. And indeed when we look at the junctions in Franconia associated squamous cell carcinoma that are shown on the left and sporadic HPV negative,
we see the same repair mechanism being active mostly on homologous non homologous end joining and MJ and that's the. The percentages are are identical. So the interpretation of this is that whenever there is a break it's being repaired just by sticking the the ends together and random fashion that results in these high number of structural variants. We also asked where these breaks occur and specifically we were interested to in knowing whether they occur at repetitive sites.
And there is some increase that’s statistically significant in sign elements, so I’ll repeat. Had higher number of breaks, but when we look at the whole genome we can see that the brakes and here I’m showing where all of this breaks out and structural variant ends are. I think when they’re being placed in the in the genome across all of the chromosomes, you can see that there is the brakes occurring throughout the genome. But you do see that parts of some of the genes are being hit multiple times. And that’s really the the way we think about it is that there is
00:43:32.428 --> 00:43:34.702 mutagenesis across and then on top
00:43:34.702 --> 00:43:37.347 of that there’s a selection of
00:43:37.347 --> 00:43:40.065 particular genes that help the tumors.
00:43:40.070 --> 00:43:43.598 Growth for example EGFR here is is being
00:43:43.598 --> 00:43:46.855 amplified and so are other sites you know,
00:43:46.860 --> 00:43:48.435 but one here.
00:43:48.435 --> 00:43:52.433 This is the EGFR and and also
00:43:52.433 --> 00:43:55.588 others the outcome of this.
00:43:55.590 --> 00:43:59.510 Is that these tumors have very high copy
00:43:59.510 --> 00:44:02.936 number variance and this is just one tumor,
00:44:02.940 --> 00:44:04.200 this is Ascot plot.
00:44:04.200 --> 00:44:08.168 copy number where total alleles
00:44:08.168 --> 00:44:10.569 obviously in the cells should be 2,
00:44:10.570 --> 00:44:13.186 the alternative allele should be one.
00:44:13.190 --> 00:44:16.200 But you can see that there are
NOTE Confidence: 0.834055263333333
00:44:16.200 --> 00:44:18.443 number of amplifications and their
NOTE Confidence: 0.834055263333333
00:44:18.443 --> 00:44:20.628 amplifications and genes that we
NOTE Confidence: 0.834055263333333
00:44:20.628 --> 00:44:23.589 all know from from tumor pathways.
NOTE Confidence: 0.834055263333333
00:44:23.590 --> 00:44:25.680 Pick three CA make cycling.
NOTE Confidence: 0.834055263333333
00:44:25.680 --> 00:44:28.776 One that’s that’s amplified in many
NOTE Confidence: 0.834055263333333
00:44:28.776 --> 00:44:32.224 of these tumors and then there are
NOTE Confidence: 0.834055263333333
00:44:32.224 --> 00:44:34.052 deletions and tumor suppressors
NOTE Confidence: 0.834055263333333
00:44:34.052 --> 00:44:37.270 One that’s that’s amplified in many
NOTE Confidence: 0.834055263333333
00:44:37.270 --> 00:44:42.276 So this is a pretty representative look
NOTE Confidence: 0.834055263333333
00:44:42.276 --> 00:44:45.844 at the at the tumor of Fanconi anemia
NOTE Confidence: 0.834055263333333
00:44:45.844 --> 00:44:49.110 patients and this is a different way
NOTE Confidence: 0.834055263333333
00:44:49.110 --> 00:44:51.978 of representing it where all of the.
NOTE Confidence: 0.834055263333333
00:44:51.980 --> 00:44:55.540 The rows are the the genes that are
NOTE Confidence: 0.834055263333333
00:44:55.540 --> 00:44:59.025 mutated and the columns are single tumor
NOTE Confidence: 0.834055263333333
00:44:59.025 --> 00:45:01.455 that we’ve sequenced from Fanconi patients.

64
And you can see that orange or amplifications and deletions in blue and multiple of these pathways are mutated in each of the genes.

And if you look at pick three CA and Mick Amplifications 54% of these tumors have Co Co amplification of these two. Oncogenes, so this is a very, this might explain part of why these tumors are aggressive but there are other reasons they they might be aggressive as well. So to just summarize what happens in franconi tumors?
We have the Franconia pathway that protects cells from creating DNA breaks when DNA interstrand crosslinks are present and if you have DNA breaks the structural variants follow and eventually all of them lead to high copy number variation in these in these tumors.

And there is also I wanted to mention there is a paper that recently came out from Jean Souliers Lab who looked at AML’s and AML’s. They also see structural variant formation, fewer structural variants, and they also see P53 pathway being abnormal. But actually P53 itself is not mutated,
it's through the MDM 4 pathway that there is suppression of P53.

So in the bone marrow similar events are happening.

Maybe it's at a slightly smaller, smaller scale, which I don’t.

Alright, understand.

And might have to do with the timing of these tumors and time to evolution of these tumors.

So knowing what we know about frankonia tumors, we then turned to sporadic cancers and we were wondering whether the structural variants that are present...
in sporadic HPV negative tumors.

I showed you that there are still some of them and they’re shown here on the right hand side. These are all HPV negative TCG head and neck cancers. You can see that there’s plenty of orange and blue. So there are quite a lot of copy number variants, not as many as in Fanconi tumors, but still. A high number.

So we were wondering whether what we find in Fanconi tumors might apply to head and neck cancer.
and sporadic and sporadic cases.

And what we decided to do is to look at a copy number variants and stratify tumors.

So now these are the TCG A HPV negative tumors and stratify them into top quartile.

Tumors with a high number of copy number variants, so top quartile, and then compare them to the low quartile.

the top 1/4 of tumors with the low copy number variants, and we could correlate the number of pack years with the level of copy number variants.
So the higher smoking history, the higher copy number variants. And when we looked at signatures that were present and in these two we could also show that the top quartile. Had about twofold increase in signatures of ID 38SBS4 and others and these are smoking associated in Dells, so that’s not surprising, right? That’s correlates with the pack history but also non homologous end joining and benzopyrene and acetaldehyde. That suggests something we we already know from epidemiological studies that smoking and drinking go together.
So we so these patients probably are also enriched for for increased alcohol exposure. So taking all of this together, we come up with this hypothesis that in sporadic cancers, the tobacco and alcohol exposure creates DNA interesting crosslinks. Obviously it creates other mutagenic events, including P53, which is an early event that then allows structural variants to occur. And Franconia pathway even though that it’s present in these in these tumors and maybe it might
be somatically decreased or maybe there are some genetic factors that are involved here, but Frankonia pathway which is largely efficient. There isn’t just enough of it and it’s overwhelmed and unable to repair all the DNA into crosslinks. It’s also important that P53 is mutated in these so you cannot depend on P53 pathway to to increase the Franconia. Green expression kind of going in gene expression patterns and all results in DNA breaks, structural variants and the same pathway leading to copy number alterations.
So taken together, you know when we look at the FRANKLINIA pathway function or if we have patients with low pathway function, they have very high levels of head and neck cancers and other squamous cell carcinomas, maybe not 100%, but if they Long live long enough, very high levels and if we have full function we are protected. However, there are a number of genetic modifiers whether it’s a. The H2CT A4DC GSTM one and others, as well as these environmental
modifiers of alcohol tobacco that is making this pathway functionally inefficient leading to higher probability of head and neck cancer. So I’ll finish and um, This is Arlene, who’s been instrumental in starting the registry. Munjung Jung has worked on a LH9A1. They work on head and neck cancers, was hugely collaborative work, and we were helped by Matthias Sanders. Working with Peter Campbell and Andrew Webster drove this work in my lab. And we had. A lot of collaborators,
clinical and patient collaborators
and other bioinformaticians
who've helped us.
And I'd like to thank my funding agencies,
tons of collaborators and
especially patients and families.
And these are some of the young adults
few years ago before the pandemic and
I know that at least five of these.
Adults have passed away with head and neck cancer and during this time.
So I'll take questions.
Thank you.
OK. That was wonderful.
For those of you who are online,
00:52:51.940 --> 00:52:55.036 please use the Q&amp;A function to bring your
NOTE Confidence: 0.858748585714286
00:52:55.036 --> 00:52:59.430 questions and I think Jeff. Jeff, yeah.
NOTE Confidence: 0.77555748
00:52:59.430 --> 00:53:01.397 Ohh uh you can have a microphone.
NOTE Confidence: 0.800516585
00:53:01.410 --> 00:53:02.418 I think it's here.
NOTE Confidence: 0.77809263625
00:53:03.990 --> 00:53:06.270 And and maybe I'll just start
NOTE Confidence: 0.77809263625
00:53:06.270 --> 00:53:07.896 with Nadia, Dimitrova asked.
NOTE Confidence: 0.77809263625
00:53:07.896 --> 00:53:10.494 Do you see evidence of extrachromosomal
NOTE Confidence: 0.77809263625
00:53:10.494 --> 00:53:12.904 DNA circles and FA tumors to
NOTE Confidence: 0.77809263625
00:53:12.904 --> 00:53:14.380 explain the amplifications and
NOTE Confidence: 0.77809263625
00:53:14.380 --> 00:53:16.588 we haven't really looked at that,
NOTE Confidence: 0.77809263625
00:53:16.590 --> 00:53:18.767 but that would be a good idea.
NOTE Confidence: 0.77809263625
00:53:18.770 --> 00:53:22.306 We do see a lot of tandem duplications
NOTE Confidence: 0.77809263625
00:53:22.310 --> 00:53:24.725 in the especially Mick and
NOTE Confidence: 0.77809263625
00:53:24.725 --> 00:53:26.772 other areas that would explain
NOTE Confidence: 0.77809263625
00:53:26.772 --> 00:53:29.090 the amplifications as well. Jeff
NOTE Confidence: 0.815526265714286
00:53:29.130 --> 00:53:30.334 yeah. So that was a great talk,
NOTE Confidence: 0.815526265714286
00:53:30.340 --> 00:53:31.608 incredibly clear and insightful.
NOTE Confidence: 0.815526265714286
00:53:31.608 --> 00:53:32.876 I really appreciate it.
NOTE Confidence: 0.815526265714286
00:53:32.880 --> 00:53:34.656 I really like that last diagram.
NOTE Confidence: 0.815526265714286
00:53:34.660 --> 00:53:36.088 I know it was meant diagrammatically
NOTE Confidence: 0.815526265714286
00:53:36.088 --> 00:53:37.720 at least the way you showed it,
NOTE Confidence: 0.815526265714286
00:53:37.720 --> 00:53:38.869 but it can’t.
NOTE Confidence: 0.815526265714286
00:53:38.869 --> 00:53:40.784 That doesn’t stop me from
NOTE Confidence: 0.815526265714286
00:53:40.784 --> 00:53:43.897 asking do you have a way to
NOTE Confidence: 0.815526265714286
00:53:43.897 --> 00:53:45.709 quantify Fanconi anemia pathway
NOTE Confidence: 0.815526265714286
00:53:45.709 --> 00:53:47.965 function on that X axis or not.
NOTE Confidence: 0.667696745
00:53:48.010 --> 00:53:49.108 That’s a really,
NOTE Confidence: 0.667696745
00:53:49.108 --> 00:53:50.938 that’s a really good question.
NOTE Confidence: 0.667696745
00:53:50.940 --> 00:53:54.126 So we we have some way of doing that
NOTE Confidence: 0.667696745
00:53:54.126 --> 00:53:56.620 within the Franconia population,
NOTE Confidence: 0.667696745
00:53:56.620 --> 00:53:58.545 so something that I haven’t talked about.
We have. The registry has been amazing and we sequence now pretty much everybody who we had. The DNA for to look for phenotype, genotype correlations and there are a lot of phenotype, genotype correlations that we can tease out and we’ve started to describe them. And the function we can at the lower end it’s hard to tease it out but you can definitely do that with number of foci with ubiquitination of fancd 2 and Frank I it’s not great I think we need a better assay for for doing that on the top end I think
when we have like what we see what

It’s really difficult to say

But as you saw from the second

part of my talk, you know,

even a little bit of DNA damage

can lead to chromosome breakage

like the ones that we induce when

we don’t have a LDH 9A1.

So I think there might be ways of of

doing better assay development for this.

So if I can

ask a question. So one of the things
we’re noticing in the virtual tumor board that we do for these young adults with head neck cancer is that they are really not at all responsive to immune checkpoint inhibition which is often brought forward as something that might not be so DNA damaging. And I wonder if you could speculate about that both from the perspective of? Sort of this global picture you’ve been talking about with structural variants, not some so many single nucleotide variants. But also, is there anything that came out in your gene signatures that would speak to? I saw NSDD one which sometimes is
00:55:58.070 --> 00:55:59.520 associated with immune exclusion.
NOTE Confidence: 0.846759041333333

00:55:59.520 --> 00:56:01.752 Are you getting a picture from the mutations of what the immune status might be?
NOTE Confidence: 0.846759041333333

00:56:01.752 --> 00:56:03.819 So from the mutations we do see deletions in.
NOTE Confidence: 0.86615868

00:56:04.720 --> 00:56:08.110 But frankly the genome instability probably has a big part in the inability to have immune response right.
NOTE Confidence: 0.86615868

00:56:08.110 --> 00:56:13.140 So we are very much interested that and we are starting to look at that tumor microenvironment in these tumors in particular thinking that it’s it will be a good model for understanding that.
NOTE Confidence: 0.86615868

00:56:13.140 --> 00:56:17.800 A message. Will say.
NOTE Confidence: 0.86615868

00:56:17.800 --> 00:56:21.664 So we are very much interested in that and we are starting to look at that tumor microenvironment in these tumors in particular thinking that it’s it will be a good model for understanding that.
NOTE Confidence: 0.86615868
00:56:45.430 --> 00:56:46.918 But it definitely looks like it would be too more suppressive, yeah.

00:57:05.190 --> 00:57:07.976 Yes. So that’s an interesting question and there is some data in the literature that implicates mitochondrial DNA damage and Fanconi function within the mitochondria. I’m not sure how the fund Fanconi proteins would get into the mitochondria, but it’s something that we have not. I’m not sure how the fund Fanconi proteins would get into the mitochondria, but it’s something that we have not. 

00:57:22.970 --> 00:57:26.148 but it’s something that we have not.

00:57:26.150 --> 00:57:29.120 Explored. It would be so that, 

00:57:29.120 --> 00:57:31.269 I’m sorry, the question was about the mitochondrial damage and whether there is increased mitochondrial damage.

00:57:34.620 --> 00:57:38.880 We we haven’t really explored that.

NOTE Confidence: 0.86615868
NOTE Confidence: 0.86615868
NOTE Confidence: 0.86615868
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NOTE Confidence: 0.887544583809524
Um, and there would be.

Well, it’s it, it would be doable, right.

I mean you just we would have to

look at mitochondria specifically.

The audio. That’s right.

A lot of these, yes.

A lot of these are the

detoxification enzymes.

Not all of them,

but many of them are within the mitochondria.

The spermine? Yep.

Not all of them,

but many of them are within the mitochondria.

The spermine? Yep.

Right. Right.

Right. Yeah. So there is a lot,

a lot that we could explore in in this

disease and mitochondrial function

definitely has been brought up as as one
00:58:27.621 --> 00:58:29.889 of the places where where we should,
00:58:29.890 --> 00:58:33.918 we should look more.
00:58:33.920 --> 00:58:36.496 There was one question,
00:58:36.496 --> 00:58:39.824 is there a bias toward either focal or
00:58:39.824 --> 00:58:42.585 ARM level copy number alterations or
00:58:42.585 --> 00:58:45.618 both equally in FA tumors when compared
00:58:45.618 --> 00:58:48.460 to HPV negative head and neck cancers.
00:58:48.460 --> 00:58:50.440 So we see, we see both,
00:58:50.440 --> 00:58:54.146 I don’t know if if we really compare them
00:58:54.146 --> 00:58:57.240 so we can say whether it’s equal or not,
00:58:57.240 --> 00:59:01.384 but we see, we see some ARM
00:59:01.384 --> 00:59:04.270 level copy number alterations as well.
00:59:04.270 --> 00:59:06.430 But majority I would say,
00:59:06.430 --> 00:59:07.950 are the focal type.
00:59:13.900 --> 00:59:18.050 OK, yes. It seems that.
Particular these metabolic enzymes. Essentially necessary to collect cells of the dehydrogenases ID. Also very common. Biosynthesis inhibitors. Do you think, well, what are your insights on a particular reason why blood cells would be more susceptible? Right. So one possibility is that they are really not more susceptible, they are just the there are fewer of them and they exhaust faster than any other stem cells, 01:00:02.120 right, so that’s let’s leave that as a possibility. The other is we know for formaldehyde. That. Very high levels of formal
formaldehyde can be formed close to the DNA during the differentiation process. So one hypothesis is that you’re having differentiation that happens during very few replication cycles, and that vulnerability of two formaldehyde is very high. Umm. So that’s a possibility in coratina sites when we do an assay for for example whether ADH two or ADH 5 or important in with human characteristics sides, there doesn’t seem to be that dependency within the fungi 2 or frank a negative. But we when we start playing with
lipids metabolism they show up. And then it makes sense because it’s the the lipid metabolism is very important in the as a barrier in correcting the sites. So some of it, I do believe that some of it will come to specific functions or a particular stem cell. Um, and I think just more needs to be done to understand, to understand that one of the challenges is that. It’s very hard to identify how much of these aldehydes are present and especially within the DNA it’s just very difficult to to quantify these. But it’s an I think it’s
a very interesting area.

OK, Ryan. Yeah, great talk about it.

This morning, your copy number, your copy number, application analysis. Have you ever

have you looked at the sequences

of those copy numbers?

Are they mutating in each copy number?

And what do you think

driving that copy number,

especially the amplification?

Do you think it’s like a DNA repair

pathway, like pull Theta

or something like that?

Yes. So let me just,
I don’t know if I left.

Oh, I can’t escape, OK.

Well, I won’t try to find slides for this.

So we as far as you mean like point mutations within the amplified regions, we don’t really see any kind of aggregation of mutations like at the junctions.

Let’s say we’ve looked at that. Um, and the way I some of these amplifications occur through probably or are consistent with let’s say that with stalling at the, at the cross link or some other lesion and reversal?
So we do see these inversions that are associated would be expected to be associated with replication. Some of them are tandem duplications and I think a nice system to figure this out is actually in a system where you have tester sequences and stalling through tester that would be one place where you could model some of these, I think. And I guess I was thinking of all these reversion mutations that arise like in Fanconi anemia perhaps. You know the copy numbers. They’re sampling different
genetic states so they can, you know, sort of revert to a.
I have a lot to say about the, the copy, not the reversions in Fanconi because we have a whole paper that’s coming soon on mosaicism in the blood and a lot of these mutations are actually somatic recombination events that are UMPD, their uniparental disomy events. So there are, yes, there are point mutations and there are other things happening you know, splice site. New splice site mutations and things like that,
but a lot of them are are full uniparental disomy through mitotic recombination. Which I think is interesting. Thank you very much. Um, for the fellows I think you’re.