The Cancer Center grand rounds.

I’m Barbara Burtness, and I’m really extraordinarily thrilled to be introducing agato 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 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 and 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
NOTE Confidence: 0.817401898
00:03:40.025 --> 00:03:43.185 generative diseases and a lot of
NOTE Confidence: 0.817401898
00:03:43.185 --> 00:03:45.660 them obviously are associated with
NOTE Confidence: 0.817401898
00:03:45.750 --> 00:03:48.720 cancer prone due to mutagenic nature
NOTE Confidence: 0.817401898
00:03:48.720 --> 00:03:51.910 of these lesions if the lesions
NOTE Confidence: 0.817401898
00:03:51.910 --> 00:03:54.150 are not properly repaired.
NOTE Confidence: 0.817401898
00:03:54.150 --> 00:03:56.712 So my favorite lesion is this interest
NOTE Confidence: 0.817401898
00:03:56.712 --> 00:04:00.083 in Cross link and I'll give you a little
NOTE Confidence: 0.817401898
00:04:00.083 --> 00:04:02.609 bit of introduction about these lesions.
NOTE Confidence: 0.817401898
00:04:02.610 --> 00:04:04.475 This is a covalent linkage
NOTE Confidence: 0.817401898
00:04:04.475 --> 00:04:06.730 of two strands of the DNA.
NOTE Confidence: 0.817401898
00:04:06.730 --> 00:04:09.508 It’s repaired by the Franco anemia
NOTE Confidence: 0.817401898
00:04:09.508 --> 00:04:12.492 DNA repair pathway and I’ll give you
NOTE Confidence: 0.817401898
00:04:12.492 --> 00:04:15.622 more of a of the mechanism of how this
NOTE Confidence: 0.817401898
00:04:15.622 --> 00:04:18.428 is done in in a bit in later slide,
NOTE Confidence: 0.817401898
00:04:18.428 --> 00:04:21.214 but the whole purpose of this pathway
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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 to rescue. Those forks so that this this intern crossing is really our model of 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 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 the cell tries 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,

any system can be affected and that’s seen in

patients who are truly null for this pathway.

Majority of patients will present with Pence,

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 this 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,
sources of interstrand crosslink. And today I'll talk about the bone marrow side. But we are very much interested in those in the keratinocytes as well. And this is an ongoing work in my lab. And then I'll talk about the mechanism of tumorigenesis and Fanconi anemia. Especially in the squamous cell carcinoma. So just to give you a flavor of how this pathway actually functions, this is a very regulated process of repair of these lesions. So we start with the ACL that you've shown you before and this
00:08:38.593 --> 00:08:41.530 is the structure that we know forms
NOTE Confidence: 0.712936646333333
00:08:41.530 --> 00:08:43.765 when the replication forks stall
NOTE Confidence: 0.712936646333333
00:08:43.765 --> 00:08:46.280 at the lesion and some of this work
NOTE Confidence: 0.712936646333333
00:08:46.280 --> 00:08:48.199 has been done in in human cells,
NOTE Confidence: 0.712936646333333
00:08:48.200 --> 00:08:49.067 some of it.
NOTE Confidence: 0.712936646333333
00:08:49.067 --> 00:08:51.090 This work was done in Johannes Walters
NOTE Confidence: 0.712936646333333
00:08:51.157 --> 00:08:53.355 lab in the Xenopus egg extract system.
NOTE Confidence: 0.712936646333333
00:08:53.360 --> 00:08:54.588 So biochemically,
NOTE Confidence: 0.712936646333333
00:08:54.588 --> 00:08:57.658 it’s a fairly understood pathway.
NOTE Confidence: 0.712936646333333
00:08:57.660 --> 00:08:59.440 What’s essential in this pathway,
NOTE Confidence: 0.712936646333333
00:08:59.440 --> 00:09:00.904 and I’ll show you the proteins
NOTE Confidence: 0.712936646333333
00:09:00.904 --> 00:09:02.359 that are involved in a moment,
NOTE Confidence: 0.712936646333333
00:09:02.360 --> 00:09:04.614 but what has to happen is that
NOTE Confidence: 0.712936646333333
00:09:04.614 --> 00:09:07.418 the the DNA needs to be broken,
NOTE Confidence: 0.712936646333333
00:09:07.420 --> 00:09:10.300 but in a very regulated way that then
NOTE Confidence: 0.712936646333333
00:09:10.300 --> 00:09:12.300 gets repaired through translesion

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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 for this mutation.

And this was Buckwald 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 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 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 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 the germline.

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,
00:11:57.880 --> 00:12:01.176 again a flavor for the for this pathway.

00:12:01.180 --> 00:12:03.676 So majority of the proteins whose
00:12:03.676 --> 00:12:06.659 genes are mutated in Fanconi patients
00:12:06.660 --> 00:12:09.540 form this large core complex.

00:12:09.540 --> 00:12:12.078 All of the colored proteins are
00:12:12.078 --> 00:12:14.352 actually those that have mutations
00:12:14.352 --> 00:12:16.545 in Fanconi patients.

00:12:16.545 --> 00:12:19.766 And you can see that this core complex
00:12:19.766 --> 00:12:22.419 is situated that this cross link as
00:12:22.419 --> 00:12:25.659 if it was identifying the the damage.

00:12:25.660 --> 00:12:26.743 It’s actually unclear.

00:12:26.743 --> 00:12:28.187 How that’s exactly done?

00:12:30.280 --> 00:12:33.928 The key to this core complex is that
00:12:33.928 --> 00:12:37.280 it has this E3 ubiquitin ligase fancl
00:12:37.280 --> 00:12:40.652 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
in making these incisions come in
and one of the important proteins
here is this SLX 4 which acts as a scaffold for three different nucleases.
And it's quite amazing how the three nucleases are associated with one protein and frank P is or a cell.
X4 is essential not only for scaffolding. But for the function of the of these other nucleases without this slex
4 none of the nucleus is actually functional within the cell.
They have full in vitro function but not not cellular function.
So it’s it’s fascinating but in
this case XPF is the one that’s making the incisions.

I’ll also mention the protein that that or disease 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 this cross link outside of S phase and we’re still very much interested in knowing how especially in the kidney and and the liver.
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 another 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 nucleases and
our lab has shown that it’s there. There is a protection against DNA to and 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 other damage. So I this is 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 I’ll say about this 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, we’ll talk about what happens 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 intrastrand 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.

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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 and definitely contribute to bone marrow.
marrow failure in Franconia 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. Franconia 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 a shore

segue and talk about a LDH 2 and ADH 5.

So a LDH 2 is necessary for removal

come from ethanol from the outside,

but also from metabolism,

intracellular metabolism.

And LDH 2 is responsible for

detoxifying it to acetate.

And a KG Patels group has described

LDH 2 knockouts when combined

with Fangy 2 knockouts.

The mouse for the first time

really developed bone marrow fell

failure and leukemia.
So the mouse model without just fancd 2 mouse model does not develop.

Bank bone marrow failure or leukemia. It has mild hematopoietic dysfunction, but 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 Fanconi anemia phenotype and there is a really beautiful paper from the Takata group that have that was published.
in 2013 which really solidified our thinking about this as a modifier of Fanconi anemia. And I also will mention that together with Chris Vakoc’s lab we showed that Somatic Aldh 2 mutation silencing in AML’s results in dependency on the Fanconi anemia. So when Chris’s lab and they are at Cold Spring Harbor did a screen and showed in number of AML’s dependency on Fanconi anemia. He called us up and asked what should I look at why are they dying? And I said just look at LDH 2 I bet it’s.
00:21:42.965 --> 00:21:44.729 that’s what it turns out to be.

00:21:44.730 --> 00:21:47.178 We just helped him a little bit but some of some of AML’s depend on on

00:21:49.974 --> 00:21:52.380 Franconia because of the of the silencing of LH2 which I think is quite interesting and has implications for

00:21:52.466 --> 00:21:56.214 some of Franconia because of the silencing of LH2 which I think is quite interesting and has implications for therapies for these AML.

00:21:56.214 --> 00:21:58.567 So then ADH five came.

00:21:58.567 --> 00:22:01.458 And that was also from KJ Patel’s work that ADH five is necessary for.

00:22:01.460 --> 00:22:04.920 I mean this is this has been known that ADH five is necessary for.

00:22:09.112 --> 00:22:13.117 I mean this is this has been known that ADH five is necessary for.

00:22:13.120 --> 00:22:15.336 that has been known that ADH five is necessary for.

00:22:15.336 --> 00:22:17.607 that has been known that ADH five is necessary for.

00:22:17.607 --> 00:22:19.728 is necessary for detoxification of

00:22:19.728 --> 00:22:22.548 formaldehyde through this through this

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. Uh new digenic human disease of LDH 2 ADH 5 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 I CL’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 Jurkat cells
which are T cell leukemia cells to figure this out.
So our hypothesis was that there
would be more of these detoxification
NOTE Confidence: 0.7890958325
path pathways in the cells and
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by understanding what that is.
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They might actually be equipped
NOTE Confidence: 0.7890958325
with understanding of what can we
NOTE Confidence: 0.7890958325
increase to have some preventive
NOTE Confidence: 0.7890958325
measures in in Fanconi anemia.
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So this is work from Munjung Jung
NOTE Confidence: 0.7890958325
who's now a a assistant professor
NOTE Confidence: 0.7890958325
at Hopkins and she was a clinical
NOTE Confidence: 0.7890958325
scholar in my lab and she did
NOTE Confidence: 0.7890958325
a metabolism focus screen.
NOTE Confidence: 0.7890958325
So this is crisper screen or that she.
NOTE Confidence: 0.7890958325
Performed in jurkat cells.
NOTE Confidence: 0.7890958325
And she took Frankie to positive
NOTE Confidence: 0.7890958325
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 membrane 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

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 two 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
of chromosome breakage if we don’t
have a LDH 9A1 Infinity 2 cells.
She also used human hematopoietic
stem cells depletedFrank 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 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 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 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. If we have triple mutants, these cells now can grow much better, presumably because we are sequestering the polyamines outside of the away from the DNA, not creating these these crosslinks. So I think this is a 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
NOTE Confidence: 0.861485793333333

00:30:58.070 --> 00:31:01.204 damage that are necessary for crosslink repair for that are necessary to be repaired by franklinia pathway and other pathways of crosslink repair.
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00:31:01.204 --> 00:31:04.389 And really adding to this idea of how the first tier of protection is necessary to protect the genome, not creating interstrand crosslinks.
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00:31:04.389 --> 00:31:07.114 And that eventually obviously is necessary for normal cell and organ function.
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00:31:07.114 --> 00:31:10.004 So with that, I’ll move to the last topic, which is cancer and Fanconi anemia, children and young adults.
And when you think about the anemia pathway, 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 BRC2. Or probably 2 mutations and they are identified early age you know they have developed these tumors within the first five years of
their life and we actually have a mouse model of medulloblastoma that is quite interesting but not ready for prime time. But the reason why we are interested again in these tumors is because we want to understand why the granule progenitor. Cells or the cells that lead to neuroblastoma or tulips tumors, why do they really need BRC 2 function? What's so special about these cells that require BRC 2 function or probably homologous recombination. So that's something that we are continuing to develop.
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 that 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. 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 the patients are diagnosed late, the survival is still poor and it’s not really decreasing and if it’s decreasing it’s just because there’s more, there are more HPV positive cancers and again the treatment can be quite horrendous for the patients and really learning from Barbara. The need is really to stratify these patients to also to identify novel therapeutics that might not be DNA damaging. So we, 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 at least 700 fold increase of tumorigenesis in the 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 work and I’ll go fairly quickly through it. This is our cohort of patients who were whose tumors were sequenced and you can see that the agent diagnosis is. On median of 31 years old,
but we have some 13 year olds with head and neck cancer that’s extremely aggressive and extremely difficult to treat since we cannot use this platin for these patients and they are radiation sensitive as well as patients although not that well 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 actually 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 gene that was mutated through point mutations, that was associated with structural variants.
So here you can see that these are number of mutations in across different cancers through TCG data from TCG data 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.
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
00:39:56.146 --> 00:39:58.636 rely on these structural variants.
00:39:58.640 --> 00:40:01.598 And this is comparable to the
00:40:01.598 --> 00:40:04.180 structural variants that we see in
00:40:04.180 --> 00:40:11.580 BRC 2 or BRC 1 tumors which have
00:40:11.580 --> 00:40:14.568 high levels of structural variation.
00:40:14.568 --> 00:40:16.440 As far as types of type of structural
00:40:16.440 --> 00:40:18.752 variance, we see number of them,
00:40:18.752 --> 00:40:20.900 inversions, all of them are increased.
00:40:20.900 --> 00:40:23.475 And if you take proportion
00:40:23.475 --> 00:40:25.020 of structural variance,
00:40:25.020 --> 00:40:27.756 actually the on the there is a slight
00:40:27.756 --> 00:40:29.839 increase of tandem duplication.
00:40:29.840 --> 00:40:32.744 So pretty much across all structural
00:40:32.744 --> 00:40:35.540 variants there is an increase.
made when the DNA when Fanconi pathway cannot function and these breaks being repaired inappropriately and it’s 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 double strand break you, it can be repaired through three different mechanism, non homologous end joining, microhomology mediated repair and single strand annealing. 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 identical. So the interpretation of this is that whenever there is a break it’s being repaired just by sticking 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 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 are 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
mutagenesis across and then on top of that there’s a selection of particular genes that help the tumors. Growth for example EGFR here is being amplified and so are other sites you know, This is the EGFR and and also others the outcome of this. Is that these tumors have very high copy number variance and this is just one tumor, this is Ascot plot. So this is a little specific copy number where total alleles obviously in the cells should be 2, the alternative allele should be one.
But you can see that there are number of amplifications and their amplifications and genes that we all know from tumor pathways. Pick three CA make cycling. One that’s that’s amplified in many of these tumors and then there are deletions and tumor suppressors like CDK and two way and P53. So this is a pretty representative look at the at the tumor of Fanconi anemia patients and this is a different way of representing it where all of the. The rows are the genes that are mutated and the columns are single tumor mutated and the columns are single tumor that we’ve sequenced from Fanconi patients.
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 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 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
00:47:07.540 --> 00:47:10.340 in sporadic HPV negative tumors.
NOTE Confidence: 0.821754858888889
00:47:10.340 --> 00:47:12.181 I showed you that there are still
NOTE Confidence: 0.821754858888889
00:47:12.181 --> 00:47:13.905 some of them and they’re shown
NOTE Confidence: 0.821754858888889
00:47:13.905 --> 00:47:15.657 here on the right hand side.
NOTE Confidence: 0.821754858888889
00:47:15.660 --> 00:47:17.510 These are all HPV negative
NOTE Confidence: 0.821754858888889
00:47:17.510 --> 00:47:19.360 TCG head and neck cancers.
NOTE Confidence: 0.821754858888889
00:47:19.360 --> 00:47:20.750 You can see that there’s
NOTE Confidence: 0.821754858888889
00:47:20.750 --> 00:47:22.140 plenty of orange and blue.
NOTE Confidence: 0.821754858888889
00:47:22.140 --> 00:47:23.700 So there are quite a lot
NOTE Confidence: 0.821754858888889
00:47:23.700 --> 00:47:24.740 of copy number variants,
NOTE Confidence: 0.821754858888889
00:47:24.740 --> 00:47:27.380 not as many as in Fanconi tumors,
NOTE Confidence: 0.821754858888889
00:47:27.380 --> 00:47:29.810 but still.
NOTE Confidence: 0.821754858888889
00:47:29.810 --> 00:47:31.289 A high number.
NOTE Confidence: 0.821754858888889
00:47:31.289 --> 00:47:34.247 So we were wondering whether what
NOTE Confidence: 0.821754858888889
00:47:34.247 --> 00:47:37.202 we find in Fanconi tumors might
NOTE Confidence: 0.821754858888889
00:47:37.202 --> 00:47:40.830 apply to head and neck cancer
and sporadic cases.

And what we decided to do is to look at copy number variants and stratify tumors. Now these are the 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 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 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 inter 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 tumors and maybe it might
be somatically decreased or maybe there are some genetic factors that are involved here, but Franklin pathway which is largely efficient. There isn’t just enough of it and it’s over is 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 Franklin. Green expression kind of going in gene expression patterns and all of this 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 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 H2CTA4DC 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.

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 also 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 are have passed away with 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 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 quantifying 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 we think fully functional pathway.

It's really difficult to say whether it's 95 or or 90%.

So we haven't really been able to do that.

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 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 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
associated with immune exclusion.

Are you getting a picture from the mutations of what the immune status might be?

So from the mutations we do see deletions in.

But frankly the genome instability probably has a big part in the inability to have immune response right.

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 will be a good model for understanding that.

So we’ll, we’ll, we’ll see.
But it definitely looks like it would be too more suppressive, yeah. Tommy.

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 explored. I’m not sure how the fund Fanconi proteins would get into the mitochondria, but it’s something that we have not. Explored. It would be so that, I’m sorry, the question was about the mitochondrial damage and whether there is increased mitochondrial damage. We haven’t really explored that.
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. Right. Right. So there is a lot, a lot that we could explore 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 we should,

NOTE Confidence: 0.876613792857143

00:58:29.890 --> 00:58:33.918 we should look more.

NOTE Confidence: 0.876613792857143

00:58:33.920 --> 00:58:36.496 There was one question,

NOTE Confidence: 0.876613792857143

00:58:36.496 --> 00:58:39.824 is there a bias toward either focal or

NOTE Confidence: 0.876613792857143

00:58:39.824 --> 00:58:42.585 ARM level copy number alterations or

NOTE Confidence: 0.876613792857143

00:58:42.585 --> 00:58:45.618 both equally in FA tumors when compared

NOTE Confidence: 0.876613792857143

00:58:45.618 --> 00:58:48.460 to HPV negative head and neck cancers.

NOTE Confidence: 0.876613792857143

00:58:48.460 --> 00:58:50.440 So we see, we see both,

NOTE Confidence: 0.876613792857143

00:58:50.440 --> 00:58:54.146 I don’t know if if we really compare them

NOTE Confidence: 0.876613792857143

00:58:54.146 --> 00:58:57.240 so we can say whether it’s equal or not,

NOTE Confidence: 0.876613792857143

00:58:57.240 --> 00:59:01.384 but we see, we see we see some ARM

NOTE Confidence: 0.876613792857143

00:59:01.384 --> 00:59:04.270 level copy number alterations as well.

NOTE Confidence: 0.876613792857143

00:59:04.270 --> 00:59:06.430 But majority I would say,

NOTE Confidence: 0.876613792857143

00:59:06.430 --> 00:59:07.950 are the focal type.

NOTE Confidence: 0.67607196

00:59:13.900 --> 00:59:18.050 OK, yes. It seems that.

NOTE Confidence: 0.39819422

84
00:59:21.650 --> 00:59:24.288 Particular these metabolic enzymes.
NOTE Confidence: 0.541407036
00:59:26.610 --> 00:59:30.520 Essentially necessary to collect cells of the
NOTE Confidence: 0.42764593028
00:59:30.670 --> 00:59:33.910 dehydrogenases ID. Also very common.
NOTE Confidence: 0.505000965
00:59:36.490 --> 00:59:38.760 Biosynthesis inhibitors.
NOTE Confidence: 0.76317863875
00:59:42.030 --> 00:59:43.734 Do you think, well, what are your insights
NOTE Confidence: 0.712604940909091
00:59:43.750 --> 00:59:45.904 on a particular reason why blood
NOTE Confidence: 0.712604940909091
00:59:45.904 --> 00:59:48.240 cells would be more susceptible?
NOTE Confidence: 0.923196818333333
00:59:50.760 --> 00:59:53.250 Right. So one possibility is that
NOTE Confidence: 0.923196818333333
00:59:53.250 --> 00:59:56.319 they are really not more susceptible,
NOTE Confidence: 0.923196818333333
00:59:56.320 --> 00:59:58.018 they are just the there are
NOTE Confidence: 0.923196818333333
00:59:58.018 --> 00:59:59.944 fewer of them and they exhaust
NOTE Confidence: 0.923196818333333
01:00:02.120 --> 01:00:04.040 Right, so that’s let’s
NOTE Confidence: 0.923196818333333
01:00:04.040 --> 01:00:05.960 faster than any other stem cells,
NOTE Confidence: 0.923196818333333
01:00:05.960 --> 01:00:07.115 so that’s let’s
NOTE Confidence: 0.923196818333333
01:00:07.115 --> 01:00:11.215 The other is we know for formaldehyde.
NOTE Confidence: 0.923196818333333
01:00:11.215 --> 01:00:15.625 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, 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, have you looked at the sequences 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 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. 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 another 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 full uniparental disomy through mitotic recombination. Which I think is interesting.