Really interesting familial syndrome of Fanconi’s anemia, which is near and dear to my heart and I am very excited to hear what he has to say since I’m not sure I’ve ever heard you talk about van company security.

Thanks, Steve.

So this is this is the quintessential genetic model of cancer, which is really what I want to tell you about and not so much that I’m going to make everybody of Fanconi expert. But hopefully convince you that not only is the science really cool, but also that this is a disease that really has importance for everybody in the room whose career touches. Both pediatric and adult oncology and so hopefully that will become clear as we move on, but

F anconi even though it’s up until recent years, has been kind of in a dusty corner of science is actually the most common inherited bone marrow failure syndrome switch Fan County is one of ‘em and this is kind of the Hall of Fame of those various syndromes.
inherited bone marrow failure syndromes are actually cancer syndromes and although rare Fanconi being the most common of these rare syndromes. Their incredible genetic models for understanding science and then how can we adapt that science to move over to perhaps some therapeutic lessons?

NOTE Confidence: 0.89746022244263

00:02:07.690 --> 00:02:38.120 So couple get into some of that today first about a little bit of a printer. Not only for Steve but for all of you. Fanconi is an autosomal recessive disease, except for one excellent variety marked by birth defects. Many of which I think will become more familiar to those of you who went through Med school and saw some of the classic pictures bone marrow failure or a plastic anemia and this peculiar hypersensitivity to DNA damage alkylating agent specifically.

NOTE Confidence: 0.882939636707306

00:02:38.120 --> 00:02:44.590 With Associated Genomic instability and leukemia and solid tumors susceptibility so.

NOTE Confidence: 0.897397518157959

00:02:45.140 --> 00:03:09.970 These patients actually look syndromic they actually look unique if you put 'em all in a room at summer camp. You’ll actually notice that they all kind of look alike. Even though it’s not all that apparent to in this picture. The other interesting fascinating part about these patients have thumb abnormalities, so they could be having this kind of aberrant looking or may be missing their thumb altogether.

NOTE Confidence: 0.909384489059448

00:03:10.570 --> 00:03:27.470 An on a radiograph an X-ray there. They may be missing their radius, which is in line developmental with the thumb and just to preempt the question later on as to why that’s the case. I have no earthly idea and no one else does either but there’s this relationship.

NOTE Confidence: 0.88367372751236

00:03:27.990 --> 00:03:34.100 Of development with radius and thumb, which is part of the genetic syndrome.

NOTE Confidence: 0.911192715167999

00:03:35.050 --> 00:04:05.580 From our vantage point it’s this trans transition from a normal healthy bone marrow to bone marrow failure over here and then with this ominous progression to leukemia, which concerns the mouse and this is a true cancer susceptibility syndrome. Now I mentioned to you, that these patients have a hypersensitivity to DNA damage. That’s really came out in the in the birth of chemotherapy. Then these patients were treated for their leukemias or solid tumors.

NOTE Confidence: 0.90643721818924
And it’s this real specific type of lesion that DNA crosslink, which can be engendered by plain old chemotherapy. But actually we stop for a second and think about it. We’re awash in DNA, damaging agents all the time whether we’re on the airplane. Cosmic radiation were breathing oxygen or exposure to aldehydes, which are part of normal metabolism. These are all Asian which will engender various types of DNA damage, but it’s really the DNA crosslink? Which.

Is the chief offending agent?

Now, if you have a little kid coming into your clinic with pancytopenia. You’ll actually even if they look completely normal because what I should tell you that a Fanconi patient can look completely normal. We always will suspect and test for Fanconi anemia, and in the case of a patient with pancytopenia and this is a test. You’ll actually order write an epic. You’ll order a chromosome damage breakage as saying, You’ll actually expose. Metaphase arrested cells to die appoxee butane or it could be mitomycin. C or could be cisplatin. Any agent that will cross link DNA and you’ll get you’ll get it definable.

A number of these broken chromosomes or stuck on chromosomes or radial chromosomes. You actually count that up and that’s actually a diagnostically and quantitatively test for sharing Coney. The other way you could do it, however, is take cells from those patients expose them to the same agents versus a wild type control or these mutant cells corrected with wild type. C DNA, whichever variety that you want and you can actually show a definable statistically significant.

So there are 22 at least 22 different jeans with any one of which is a mutant BI Allelic Lior on one allele with excellent variety. Thank be, but any defects of any of these jeans will lead the Fanconi anemia, not going to go through all of them in any detail but just to point out a couple of salient features first of all.
Uh there's at least 8 of him, which coalesce into a core complex of proteins with E3 ligase activity, which I'll come back to momentarily. The other thing is that?

Many of them are actually breast cancer jeans, and it was the cloning of BRCA2. Two that once it was appreciated that the Frank D1 group is actually BRCA2 which really brought this disease out of sort of the long forgotten neurons of everyone working in DNA damage repair and into mainstream cancer biology because it was clear that these proteins are part and parcel of homologous recombination.

So we'll get back to all those concepts in a minute. But just to sum up. This is yes of America. Failure syndrome, culminating in leukemia risk. But these patients if they're lucky enough to grow up to adulthood, get solid tumors. A lot of the head and neck of the genito urinary tract as well. So if you let the current therapy is you transplant. These patients they grow up and then they were getting a whole host solid tumors that we have to worry about.

And Mark biologically by this DNA damage hypersensitivity phenotype so this is the biology kind of summed up in one side and although I’m going to make you sit through another few minutes of biology 22 different protein products which have a core complex, which puts the ubiquitin on the central complex called ID, which then.

Brings about a whole bunch of other proteins into play and you see here by Immuno florescence after DNA damage this diffuse staining becomes punctate.

This is a faint D2, one of these proteins here and this is what a lot of DNA repair proteins. Do they respond to damage they come together into repair foci and then they pulled together. Other proteins and that’s really what’s going on here and you can see this is a Hall of Fame of DNA repair listed here. Rad 51 bracket 2 bracket. One and it’s turned out that all of these are actually bona fide Fanconi jeans.
So just going to give you 3 little research vignettes that are going on in the lab to give you a flavor for both what’s going on in the basic science side of what we do, and then how we’re trying to pull things over to a more therapeutic applied.

NOTE Confidence: 0.881092607975006

Project that kind of takes some of the biologic lessons that we learned how can we adapt them case, number one this is an overview of homology directed DNA repair and this is actually work we did with Patrick’s ongoing. Patrick sung that focuses on bracket. One Bard, one bracket. One Bard, one proteins, which are intimately involved with homologous recombination, a coordinated set of steps, which take from.

NOTE Confidence: 0.900857865810394

Go from DNA lesion to recruitment of the homologous chromosome invasion of that chromosome read off of that chromosome and restoration of a normal DNA Helix and it’s really formation of the central DNA repair intermediate. the D loop? Which is formed by the invasion of that homologous chromosome, which is the hallmark of this process, so Bracken one. Bard one probably don’t need to tell anybody hear what this is all about Bracco, one being the first describe breast cancer gene but.

NOTE Confidence: 0.891306698322296

Little did any of you in the room know before today, the bracket. One is a bona fide. Fanconi Gene and so this is what really pulled along with brackets who pulled the whole field into mainstream cancer biology and again not overload. You in 30 minutes with all the intricacies of what we do in the lab this is merely.

NOTE Confidence: 0.923939228057861

We can actually make these proteins in vitro? We can make bracket one. Bard one in million dollar amounts we can actually in a coordinated.

NOTE Confidence: 0.914224147796631

We can incubate radioactively labeled DNA with rad 51. The homologous recombination enzyme along with the bracket complex and you can actually reconstitute. These homologous recombination. Intermediate’s in vitro as evidenced by this slower. Mobility D loop on a gel and you can see that by adding.

NOTE Confidence: 0.841466546058655

Brad 51, Braca One Bard, one you can actually create these D loops as measured here all in vitro and.
By doing that you can then reconstitute whole model and you can go all the way from double strand break all the way to restored double Helix and demonstrate that bracket. One Bard ones involved in multiple levels of these, including the processing of the double strand break a loading rad 51. The recruitment of the homologous chromosome and eventual restoration of the double Helix so another little vignette.

This is actually taking clinical data and then going back to basic biology. This was actually data that I found out about just by going to the precision. Madison tumor board here at yell and this was actually ahead in that case, something adult who had two mutations in the facts.

Sorry about that in the faint D2 gene one being germline germline point, mutation and then this patient had acquired deletion 6 me know acids in Frankie Two and a conserve region of the protein.

So we decided to work this up a little more and we actually did a mass spectroscopy experiment of Frankie to either wild type or missing that domain and found a whole bunch of different proteins that were coming down.

In that particular domain and one in particular, caught our eye and that was the blooms syndrome. He Lakas, so blooms, which hopefully many of you have heard of is Bloom’s a wreck you helicase. It’s actually very much involved with malgus recombination defects and blooms. Helicase results in chromosome aberrations and this is another true cancer susceptibility syndrome.

So we actually further map this little region that was missing with in that particular case of head and neck cancer and we actually were able to identify one particular residue. The 822 loosing which one mutated to alanine resulted in the aggregation of interaction with Frank D2, so in this situation you see thank D2 being pulled down by in Munich precipitation here. Bloom syndrome protein being pulled down here, except in the case of this point mutant so.

By all accounts seems to be functionally a very important domain. We can further show that this is functionally important
by expressing this particular Frank D2 Mutant mutant cells. So we have these baseline mutant cells, which driver mind you again or hypersensitive. the DNA damage. If we provide the Wild Type C DNA back. These cells are thus restored for wild type resistance to minimize and see. But if we express this point, Newton to Faint D2.

NOTE Confidence: 0.87512594461441

00:14:24.290 --> 00:14:55.200 There’s still cells remain hypersensitive to DNA crosslinkers. We can actually see the phenotype biochemically as I mentioned to you. Thank the 2:00 becomes ubiquity nated by a pond DNA damage it. So you can see here in the Wild Type Lane in either of this 561 K to army, or the 8:22 meeting. The ubiquitination event is actually diminished so biochemically as well as from a cell biology point of view, we can actually prove.

NOTE Confidence: 0.911216676235199

00:14:55.200 --> 00:15:14.060 That this patient tumor derived DNA sequence information is actually functionally important so we’ve actually done this on numerous occasions to try to actually show that these mutations derived from clinically clinical cases are actually functionally important.

NOTE Confidence: 0.8765949010849

00:15:14.630 --> 00:15:44.980 Then finally we can actually show that I D2, the ID too complex composed of Frank. I'm Frank D2 actually has functional importance. So we know that Bloom can actually cause the solution. The unwinding of a double strand substrate and you see double strand goes down to single strand here by addition of recombinant ID to protein you can see eventual complete virtual complete inhibition of that process.

NOTE Confidence: 0.897670030593872

00:15:44.980 --> 00:16:11.070 And that point mutant without showing you all the data. Here, the point. Newton that we should be functionally important in the previous slides actually is completely non functional in the South side, so by biochemical and cell biology assays were able to show that this is a particular important region of the think D2 protein both clinically as well as biologically in these assets.

NOTE Confidence: 0.887731194496155

00:16:11.720 --> 00:16:44.790 So it appears that Bloom has we know that Bloom has function in the processing of the Replication Fork and so the Replication Fork encounters damage and it stalls and then a whole bunch of other proteins are are recruited because there has to be protection of this replication for during the process of repair and so wild type in the wild type situation. We have bracket. One bracket soon. Rather, 51, protecting this fork with the addition of ID 2 in bloom, but when Bloom is there alone.
That’s all I’m wines in an unregulated fashion and nucleuses comment and degraded. So when you have proper protection proper modulation. Abloom helicase you’re able to protect that Replication Fork.

So this is not the only scenario in which the Fanconi Pathway intersects with other DNA repair pathways. Bloom being this particular example. We also have work we’ve done in mismatch repair proteins. Interacting with Frankie to an actually other DNA repair proteins like XPF are actually bona fide Fanconi Jeans as well so.

It’s clear that Fanconi anemia jeans don’t act in isolation and there’s a whole web of network of DNA repair pathways that come together to repair complex DNA lesions like a DNA cross link.

So this is kind of an overall view of what actually goes on in my lab, but this is sort of an overall view of what happens during DNA repair, which really can be thought of as a signal transduction event. Initial processing of a DNA lesion through nucleotide excision repair and then activation of the central Fanconi Proteins D2 Frank I to coordinate downstream processes such as replication and repair and then transcription a whole another set of projects, which I.

Don’t have time to tell you about today and then finally how can we kind of take these biology lessons and apply them to therapy and this is something we’re very much interested in for a few reasons. First of all for all the world. Fanconi anemia, looks like Milan dysplasia and in fact acts like Milan dysplasia, Fanconi’s preleukemia syndrome.

And and it may not be all that it may not be completely empirical that that’s the case ‘cause. We know that in the literature. There are actually Frankel. One of the subgroup when subgroup of Fanconi fantail mutations, which showed up and MDS cases.

We’ve also found a connection in work. I’m not going to tell you about today between slicing factors, which are known to be multiple ones mutant in NDS an fanconi proteins. Terms of interaction in sporadic cancers. There’s actually a wide range of cancers, which can reported with sporadic fan counting gene mutations. Jeffs car actually looked at this for us and had noted about 15 to 20% of all the positive sequence in cosmic.
Has Fanconi Gene Mutation so with the caveat. Of course, that we don’t have specific data that all of those mutations are functional have functional impact, except for the ones that we’ve actually looked at directly, but these are certainly this is certainly a sizable percentage for a rare genetic syndrome. Just remind you. These are not patients with Fanconi anemia, and then finally Bracken Mutant tumors, which remembering again that bracket.

If your bio lyrically mutant for bracket jeans. You have Fanconi anemia by definition bracket to bracket. One part of the biology of FA and of course, part inhibitors are an important new part of the cancer therapy armamentarium.

So we wanted to look a little bit more broadly at the scope of Fanconi Jean Van Coney Biology and how that could be applied more across this broad swath of tumors that are potentially mutant in Franconi Jeans, defective biology. So we actually took a genetic screen using an Sir in a library with the goal of could we eventually identify pathways? Which, when targeted could treat non Fanconi patients with Fanconi Gene.

Tumors muting Timbers or Fanconi patients with head and neck tumors for example, really have very few options. It’s one thing to do a bone marrow transplant preemptively and prevent leukemia. But there’s nothing. You can do to prevent a Fanconi patient from getting ahead in that cancer as an adult.

And so the idea here is to find a knockdown of a gene genetic pathway, which causes wild type to have much greater viability over the mutant and that’s really the definition of synthetic lethality. I won’t go much into the suppressor screen. It’s up just to show you how we set it up and we did. This set the W campus in high throughput fashion and got a relatively limited number of interesting hits, including PL K1. We want which in particular are linked because we want as a substrate appeal, K1 and they actually have inhibitors.

In clinical trials that you can take right off the shelf and we’ve actually used in the lab and in particular, and this was done in a D2 mutant background part did not show up as a hit. And so I think the lesson here and we’ve been able to document this repeatedly in the lab just because you work on a DNA repair pathway doesn’t mean that those particular
cells when they are going to be sensitive depart inhibitors part inhibitors work
clearly in Abraka mutant background.

NOTE Confidence: 0.849648356437683

00:22:06.320 --> 00:22:09.200 But not another Fanconi genius and backgrounds.

NOTE Confidence: 0.888269484043121

00:22:09.770 --> 00:22:24.060 So I think that was a lesson that we’ve learned
and just to show the data on we one. When we knock it down. We can show
that there’s significantly lower viability in our mutant cells versus wild type
cells.

NOTE Confidence: 0.877908051013947

00:22:24.900 --> 00:22:59.060 And in this case, we actually functionally evalu-
ated another mutant that we obtained from the precision. Medison tumor board
in another head in that cancer case. This being a Mutation Fanque and here
you have wild type franquet or that mutant version expressed here ubiquitina-
tion of Frank D2 here in the wild type, but not in the mutant and that’s a
readout of Fanconi Mutant Biology. Appropriately, the wild type gene corrects
the mitomycin C sensitivity here, but the.

NOTE Confidence: 0.877703011035919

00:22:59.060 --> 00:23:14.930 Mutant allele does not an you can notice here
that the mutant cells. Are we one sensitive. We want inhibitor sensitive while
wild type. Cells are relatively resistant an all of these cells have equivalent parp
inhibitor profiles.

NOTE Confidence: 0.859083652496338

00:23:16.000 --> 00:23:47.770 And finally we’ve actually developed some urine
Lynn Thom’s from a faint D to knockout mouse, which were actually using as a
tumor model in lab and we have a hit lymphoma versus a knockout limp comma
and we can actually show. There’s absolutely no effect of We want inhibitor
on the hits, but the wee ones show. Some retardation of growth in as a single
agent. So were these are just been getting out experiments that were going to
try to lay.

NOTE Confidence: 0.874388337135315

00:23:47.770 --> 00:24:14.480 The basis for using this eventually patients who
actually have mutations in these particular jeans. So our pipeline is verifying
these pathogenic mutations in sequence data looking at tumors obtained from
that got mouth D2 as well as fix C and utilizing these synthetic lethal data to
try to support their use eventually in some kind of clinical trial.

NOTE Confidence: 0.917275547981262

00:24:15.430 --> 00:24:28.020 So with that this is just all the people in the lab
both present and past who contributed to this work and just a partial list of all
the various People we work with here it yeah.

NOTE Confidence: 0.879991412162781

00:24:28.630 --> 00:24:31.840 With that happy to take any questions.

NOTE Confidence: 0.803381085395813

00:24:36.270 --> 00:24:46.500 It’s a good way to get out of get out of work that you’re finding.

NOTE Confidence: 0.82148003578186

00:24:47.210 --> 00:24:51.430 Mutations in their tumors are they are, they homeless I guess they’ve Genomic.

NOTE Confidence: 0.840952754020691

00:24:53.710 --> 00:25:12.770 No so the first case, I told you was a patient with a germline mutation. In one allele and the second allele was acquired the second case both alleles were actually acquired so it’s a mixture almost certainly.

NOTE Confidence: 0.861467361450195

00:25:14.060 --> 00:25:46.130 You approach your patients who are developing leukemia. Other tumors in the kids differently. I know that for transplants. They have to have reduced doses. For example, the overarching motivation is really to get patients transplanted early, but not too early where you create such more ability from the transplant. So so the doses are markedly reduced as you’re pointing out, they’ve also included.

NOTE Confidence: 0.821545839309692

00:25:46.130 --> 00:25:52.470 Asian cycle therapy in which have remarkably low toxicity in patients with and that’s

NOTE Confidence: 0.86960256097009

00:25:53.000 --> 00:26:14.800 Flair when put Arabic came on the scene. Patients with Fanconi, who are getting transplanted went from minority surviving 23 Force 80% long term survival, but of course, it’s a good problem to have I guess so the fact that they grow up? And now we’re getting ahold of solid tumors?

NOTE Confidence: 0.892112910747528

00:26:16.920 --> 00:26:47.050 The sweet spot is like between 5:00 and 10:00, so there, I would say that the vast majority will not get leukemia till after the age of 10. So when we started seeing their accounts kind of drifting down. Assuming then there’s going to be upward pressure on their bottom arrow to keep up that’s kind of when we try to get them transplant give any sense for what percent of thin cone is obviously it’s kind of a possible question. I guess are not recognized when we see these young adults with other States and then we screen.

NOTE Confidence: 0.875781834125519
Not to have eikones I think the percent, is hard to say, but there’s definitely patients. There I get called over by the head and neck team couple of times a year now and there’s clearly about 1/3 of Fanconi. Patients do not look like the textbook and so, yeah, I got called over by head in that team and usually to see a patient who is had inordinate amount of toxicity from the chemo and so.

We identified a 50 year old few months ago with further discussion. Turned out how to family history that he was the product of a first cousin marriage.

So that actually didn’t take too long to tease out.

Well, you know, we also don’t even know I mean, most of the jeans. Most of these Fanconi jeans have no identifiable functional motives. We don’t really know their specific function. So it’s a little hard to know to make predictions based on that. They have surprisingly few defects. They do not have spontaneous bone marrow failure. They don’t get spontaneous cancer the tumor.

Model I showed these P 53 Hut background so you need that in order to get them to elaborate in cancers.