Good morning. So for those of you who either can’t see me or don’t know me, I’m Eric Weiner and I’m really pleased to be here to introduce Kathy Wu. This is the inaugural lecture of what we hope will be a new series and many of us in the Cancer Center spent a lot of time thinking about how we want to do conferences. And we looked at attendance and we looked at who goes to what and ultimately came to the decision that Grand runs as it was, which is now trying to be in person as much as possible,
was largely attended by clinically oriented people in population, scientists and people who are otherwise looking for lunch and or breakfast. And that there was really a need for a conference that focused a bit more on translational and basic questions. And so after some thought, a small committee of people that included Katie Politi and Megan King came up with the idea of trying a conference like this on a monthly basis. And this is the first of those. So I'm really pleased to have Kathy Wu here. I've known Kathy for many years. She was a fellow at Dana Farber and of
course it’s still with Dana Farber when I was a substantially younger attending. And in fact we worked together in clinic, yes, briefly. She dabbled a little bit in seeing a patient with breast cancer or one or two. And so I’ve known Kathy now for 20 plus years and Kathy has built really a phenomenal career at Dana Farber. Her own interests are broad. I learned last night something I didn’t know before, which is that she even had an interest in sickle cell disease and therapeutic approaches to sickle
cell disease way back when, and ultimately decided that some amount of focus was needed. And her interests have really focused on immunotherapy and Col. and beyond that, the development of vaccines and tumor specific vaccines. She is presently a Professor of Medicine at Harvard Medical School and the chief of the Division of Stem cell transplantation and cellular therapies at the Dana Farber Cancer Institute. So it’s really a pleasure to have you here.
We’re all looking forward to your talk on largely on CLL.
And thanks so much for coming.

I will just say that a small group of us had a great dinner with Kathy last night.
And in addition to being a great scientist, she’s also just a delightful person to have dinner with.

Well, it’s really an honor to be here and happy New Year, Happy Snow day.

Thank you everyone in the room for trudging in this.

It’s really great to see you in person and also to all the folks out in Zoom.
I hope this is a successful series because I do think that the intersection between the clinical and the basic and really kind of being able to look at the translational opportunities that are afforded by the patients that we treat in the study are immense and so very rewarding. So and as Eric said I do have many, many different interests. I think that’s a hallmark of a happy MD. So like we’re interested in a lot of things and thank you for giving me the opportunity to maybe share some of the work that we’ve been doing in CLL Genomics. OK.
So we'll start. Let's see here we go. Disclosure slide, OK, I thought I'd start here, which is you know I think just a a challenge to all of us in the cancer community whether or not we study CLL or not is really the challenge of tumor heterogeneity and evolution. This has really been kind of understood for quite some time now, made ever more clear through all the genomic studies that have been out there. But we know for sure that cancer is a heterogeneous population,
for better or for worse.

Unfortunately, by the time that we are diagnosing patients with cancer, we’re really here at the time of escape where there’s already so many different resistance mechanisms that have really come into play. We also increasingly know that this is not happening in a vacuum, that there’s an interaction with the host immune system.

But again, by the time that we’re seeing patients,
there's so many different immune based escape mechanisms that are at play as well. And so a lot of the questions that I think as a field that we're really interested in asking is not only this question of tumor heterogeneity and evolution, hetergeneous tumor microenvironments are T cells there at the right place at the right time? How are we responding to diverse immunotherapies? And then what is the role of a tumor antigen in shaping the tumor response?
I'm not going to talk today so much until the very, very end, but this is a very large area of interest in my group. And as I said, I'm going to focus on chronic lymphocytic leukemia, which honestly the questions that I'm asking could be in any sort of tumor system. But CLL really has a lot of very unique features about the disease that have made it exceptional for the study of genomics. First, in a small tube of blood you have very pure tumor that can is readily
00:06:21.365 --> 00:06:23.235 accessible directly from the patients.

00:06:23.240 --> 00:06:24.997 The other thing is for a cancer, it’s quite indolent.

00:06:26.488 --> 00:06:28.910 And so what that means is that we really have really long disease histories of patients.

00:06:30.401 --> 00:06:32.117 We can really take snapshots in time and study evolution in real time along with the patient.

00:06:35.018 --> 00:06:37.733 And so for some time now, our group together with colleagues in the Boston area, we’ve actually had this program where we’ve been trying to study the link from genome to phenome.
How can we genomically characterize CLL, how can we understand the clinical course in response to therapy and then how can we also functionally characterize the pathway dependencies and really thinking about how we can do better.

So what I'm going to talk about today is update the group on recent genomic studies and CLL driver discovery bid on our efforts in looking at tumor heterogeneity in our CLL GEM models.

And then just a few perspectives of where we're going next in terms of the genomics.
indolent disease, it’s typically marked initially by what we call watch and wait. So there can be a long lead time, but ultimately with treatment there can be cycles of recurrence that happen with shorter and shorter intervals, much like what we see in other tumors. I think a question that has always fascinated people in this field is how do we understand who progresses faster or slower? And what I mean by that is that there are some patients who succumb to their disease within two years.
There are others that can have a little bit of therapy here and there go on for more than 1015 years. So why is that? What are the differences between the patients despite all their cells looking relatively similar under the microscope? And so for since forever there has been a long effort to try to understand those markers that we could use to distinguish amongst patients initially looking at clinical features, protein markers. But I would say over the past 10-15 years since there’s been the next generation
sequencing that’s been available to us, there’s really been an explosion of knowledge in terms of the genetic alterations later on top of that, the transcriptional alterations and even the epigenetic alterations so that we can understand what's going on. This slide really summarizes a lot of work that has been done since next generation sequencing has come upon us. I would say that the first studies in genomics arrived around 2010, 2011. We were among the first to describe mutated SF3B1.
So a splicing factor that kind of came out of the sequencing. No one had until then kind of put altered splicing and lymphoid malignancies together. There’s been large scale studies in looking at clonal evolution. So again CLL was one of the first places that studied really this kind of concept of clonally evolving subpopulations. And then and you can see initially our studies were about 100 patients and then about 500 patients per cohort. What I’m going to describe for you now is our recent work trying to put together all of these different....
studies together so that we could get a cohort of more than 1000. I want to say that during this time that we’ve kind of performed these sort of genomic studies, there has been vast changes in the therapeutic landscape of CLL therapy. So whereas previously it was very standard to get chemo immunotherapy, I would say that in the same time frame that I’m speaking there has been the introduction of targeted inhibitors of BCL, of the B cell receptor signaling and also introduction of immunotherapy.
So the really big changes, you know as we start to think about the genomic lesions. So how do we build an integrative CLL map? Well, we joined forces between our colleagues in North America but also with our colleagues in Spain and Germany and together collected cases for which there was exomes, genomes, RNA sequencing and methylation profiling. And there was a nice overlap of these different platforms in several hundreds of patients samples that we collected. And this is a kind of a
00:10:58.706 --> 00:11:00.153 intimidating commute plot,
00:11:00.153 --> 00:11:02.064 but I think it just speaks of
00:11:02.064 --> 00:11:03.919 a number of different things.
00:11:03.920 --> 00:11:06.118 First, I want to acknowledge the young
00:11:06.118 --> 00:11:08.757 people who were the leaders of this project.
00:11:08.760 --> 00:11:10.998 So I had the pleasure of working with
00:11:11.000 --> 00:11:14.280 Binyamin Nisbacher and Ziao Lin and
00:11:14.280 --> 00:11:16.943 Gaddy Goetz’s group computational gurus
00:11:16.943 --> 00:11:19.143 and then Cindy Hahn from Dana Farber.
00:11:19.143 --> 00:11:22.077 and then Ferran,
00:11:22.080 --> 00:11:25.048 Awesome lymphoma oriented fellow
00:11:25.048 --> 00:11:27.274 and then Nadeau and Marty from the group in Spain,
00:11:27.280 --> 00:11:30.880 the Spanish CLL group in Barcelona,
00:11:30.880 --> 00:11:33.160 Barcelona.
00:11:33.160 --> 00:11:33.516 Barcelona.
And then when we looked at these more than 1000 patient samples, in fact we were able to have greater sensitivity. In the magenta are all the new drivers that we identified. So each row is a driver alteration, each column is a different case and what you can see is in fact there is a list of recurrent alterations, but a long tail. You can see that a lot of our discovery is down here at the one 1% or less level. So many different sort of driver alterations that we had greater
sensitivity to identify because of the increased power of our cohort.

Just to make a really beautiful Long story short,

we were able to double the number of CLL drivers that we were able to identify previously.

There were about 10% of patients that we couldn’t account for.

There wasn’t any sort of driver alteration that we could point to that was this is the reason that they have CLL and we’ve been able to close that gap so that there’s only by now 3.8% that we
can’t account for the two large categories of CLL that are well known in the clinical arena on the basis of their immunoglobulin locus, the mutated and unmutated IGHV. We finally had enough power to actually break those two groups apart and look at them separately and they really look like very different diseases. It highlights the diverse trajectories of clonal evolution. So maybe by virtue of where you start as AB cell, maybe there’s a path of different
paths of least resistance that gets you to where you’re going to be.

And what was super interesting is that at least for the unmutated CLLS, their source of heterogeneity was genetic.

There was a lot of putative drivers in this unmutated group, but in the mutated group, relatively few drivers, but a lot of transcriptional diversity.

So really a different path to achieving that type of heterogeneity.

And then what I want to show you is that when we looked at the expression,
you know,

Benjamin was able to identify what he called E CS expression clusters.

And then the nomenclature here is some of them were enriched for M for mutated, some for unmutated.

And what you can see is that it actually breaks down the group’s more or less based on mutated or by their epigenetics.

But you can also see by the fact that there’s two colors within each column that there was contribution from both mutated and unmutated to these different expression clusters.
One thing that was really interesting is that by the yellow asterisks we could see that certain genetic alterations actually also segregated together with these expression clusters, suggesting that they were a cohesive entity each of these different expression cluster group. For example trisomy 12, which is a very well known cytogenetic abnormality associated with CLL, but for which there’s great heterogeneity in kind of the behavior of those trisomy twelves. They actually split out into two groups.
one that’s in a more predominantly unmutated group,

another in a predominantly mutated group.

And this maybe provides us with some understanding for why some... samples with the same sort of cytogenetics might behave differently.

when Benjamin started to look at these different expression cluster groups, they actually did display different clinical outcome because we had very long clinical. These were also clinically annotated samples as well. And this is just kind of the
final data slide related to this, which is indeed when we kind of breakdown the samples based on their classical clinical group, based on the expression clusters, whether they were concordant or discordant to that classification, we could actually see differences in their clinical outcomes suggesting that our expression cluster system was actually increasing the accuracy of what we’re trying to do in terms of prognostication. So we’ve been really excited to, I mean this is really,
00:15:47.440 --> 00:15:49.064 this was really a Tour de force
NOTE Confidence: 0.926483031666667
00:15:49.064 --> 00:15:50.438 effort to bring together not
NOTE Confidence: 0.926483031666667
00:15:50.438 --> 00:15:51.993 only all these different groups
NOTE Confidence: 0.926483031666667
00:15:51.993 --> 00:15:53.480 together and their expertise,
NOTE Confidence: 0.926483031666667
00:15:53.480 --> 00:15:57.000 but also to layer on all of these
NOTE Confidence: 0.926483031666667
00:15:57.000 --> 00:15:59.440 different genomic layers to kind of
NOTE Confidence: 0.926483031666667
00:15:59.440 --> 00:16:00.988 identify unique molecular subtypes.
NOTE Confidence: 0.926483031666667
00:16:00.988 --> 00:16:04.079 And I do want to say that this,
NOTE Confidence: 0.926483031666667
00:16:04.080 --> 00:16:06.072 these studies were samples that were
NOTE Confidence: 0.926483031666667
00:16:06.072 --> 00:16:08.798 collected in the era of chemo immunotherapy.
NOTE Confidence: 0.926483031666667
00:16:08.800 --> 00:16:11.635 We are actively trying to look now
NOTE Confidence: 0.926483031666667
00:16:11.635 --> 00:16:14.753 how these relate to the modern era
NOTE Confidence: 0.926483031666667
00:16:14.753 --> 00:16:16.819 of targeted inhibition and we also
NOTE Confidence: 0.926483031666667
00:16:16.819 --> 00:16:18.450 are interested in in trying to look
NOTE Confidence: 0.926483031666667
00:16:18.506 --> 00:16:20.324 at whether or not the different
NOTE Confidence: 0.926483031666667
00:16:20.324 --> 00:16:22.430 molecular subtypes have differences
in therapeutic vulnerabilities.
Now I think you know as we’ve gotten better with our therapies we we always have to kind of reckon what is the area of most unmet need. And I think right now clinically for CLL there are so many different therapies available, but we are still really faced with the conundrum of Richter syndrome. This is really it’s a rare, it occurs in five to 10% of patients with CLL but it is a transformation of a small indolent histological type into a high grade lymphoid malignancy.
90% have Histology similar to diffuse large B cell, large B cell lymphoma. The majority are clonally unrelated. We know that because if we follow their immunoglobulin, the clonal immunoglobulin, we could see the same in the patient. Shown here is a micrograph that shows a sample where you can see the coexistence of these two entities within the same sample and you can see the really big kind of histological differences. These are the patients that we typically say...
much more that we can do for you.

And it’s been very difficult to understand molecularly much about this entity because there’s been limitations of tissue sampling and and it’s really based on morphologic diagnosis. There’s been a lack of markers and understanding of genetics and for a blood based malignancy like CLL, Richter’s is really like a solid tumor. I mean, this is really so unlike what I said before where there’s ease in kind of having blood draws here. We have to get biopsies often FFP specimens in order to study.
And and this has not been, not been easy.

But I would say that over the past couple years that because of the availability of all these nice genomic platforms, there’s been really an explosion of new studies that have come out in the past year and a half. And at the same time there’s been modeling that’s been done trying to really put our attention to how we can generate mouse models, whether they’re PDXS or Gem models to try to understand this. And there’s been actually a lot of progress in understanding the genome that the genetics looking at the
epigenetics and the transcriptomics.

And so what I’m going to demonstrate for you in the next couple slides is some of our efforts in this area.

This is really work that’s been led by Aaron Perry who is now a new junior faculty member at the Dana Farber in the lymphoma group, Roman Guiz who’s part of Philo back in France and Ignot Lechner who is now a junior faculty member at BU. And what we tried to do was assemble a nice paired matched cohort.
but antecedent CLL matched together

with the Richter's where we could track evolution in time across these patients.

This was about 50 patients

that we collected samples on.

I think the point of emphasis that I want to show you on the left side here is the CLL course,

the green is the different lines of therapy.

On the right side is the Richter's

side here is the CLL course,

the green is the different lines of therapy.

On the right side is the Richter's

and I want to show you that on the

left side it's years whereas

left side it's months.

So this kind of gives you a sense of kind

of the the time course of these patients.

The black dots are the different samples
that we collected on the CLL course. The yellow here is the Richter diagnostic sample. Unfortunately, there’s a lot of red here, which is that the patients did succumb to their disease. There’s a number here with black arrows that are living. For the most part, these are patients who. We received therapy and then went on to stem cell transplant and really did a complete overhaul. So we obtained egsgomes on most of
These patients also had some genomes, RNA sequencing and single cell sequencing data. But I want to point out to you that you know a lot of these studies are really quite different. I think that the conundrum that we’ve met with Richter’s is that it’s really two malignancies in the same sample. So how do you pull apart the genomic contributions of one versus the other. And for that we had a come up with a computational approach that was quite challenging, but we were able to succeed where we really optimize the copy number.
00:20:44.139 --> 00:20:46.656 analysis to deal with FFPE artifact.
00:20:46.656 --> 00:20:49.932 We had a number of different filters
00:20:49.932 --> 00:20:52.582 that allowed us to kind of increase
00:20:52.582 --> 00:20:54.292 the sensitivity of our analysis
00:20:54.292 --> 00:20:56.110 and deal with contamination of
00:20:56.110 --> 00:20:58.480 whether tumor in the normal or
00:21:01.800 --> 00:21:02.536 the reverse.
00:21:02.536 --> 00:21:05.112 As I said the artifact from FFPE.
00:21:05.120 --> 00:21:07.532 And then we were able to put in our
00:21:07.532 --> 00:21:09.380 algorithms that allow us to identify
00:21:09.380 --> 00:21:12.495 clones and then also establish phylogeny.
00:21:12.495 --> 00:21:14.000 So at the end of the day,
00:21:14.000 --> 00:21:16.086 we were able to separate out the
00:21:16.086 --> 00:21:17.600 contributions of the CLL clones
00:21:17.600 --> 00:21:18.720 compared to the Richter’s clones.
And in doing so then we could look at start to look at phylogeny and understand which branches were CLL versus Richter's and look across time.

So again, Long story short, I think one of the questions that has been asked in the field is it is Richter's, is it a distinct entity, is it similar or is it different from the Novo DLBCL? And here we had the advantage of being able to access older data of more than 300 samples of lymphoma that our colleague market ship had collected.
And you can see across the purple on the top that the Richter’s really stand different. They’re you know separately from DLBCL and so the clonally unrelated Richter. These are the few samples here do appear to be like de Novo DLBCL, but the vast majority, the clonally related stand separately among the Richter’s itself. We were also because of all the genomic alterations that we found we were able to also perform unbiased clustering and discern that there’s
actually it appears to be molecular subtypes within Richter’s itself and these TP 53 has long been associated with Richter’s but we can see that there’s different flavors. So this one here has enrichment in whole genome doubling this group. Here RS3 has Co occurrence with Notch one also deletion 15 Q which covers MGA which is effects Mick and then RS5 also has Notch one as well wild type Notch one and a lot of copy number alterations. There were also two other subtypes that did not have TP53K Ras S Pen, Notch one together with
00:23:25.656 --> 00:23:28.712 Trisomy 12 and also SF3B1 with EGR Two.

00:23:28.712 --> 00:23:30.802 And again these different subgroups appear to have different clinical behavior where the ones that have

00:23:35.400 --> 00:23:37.759 TP 53 seem to have worse prognosis.

00:23:39.307 --> 00:23:41.405 Now what is the meaning of kind of trying to look at all these different genomic alterations?

00:23:44.589 --> 00:23:46.836 Well one thing we realized is that maybe we could harness all of this and actually look to see this,

00:23:48.720 --> 00:23:51.600 whether this could help us devise a non-invasive approach to identifying Richter’s and getting us to earlier detection.

00:23:54.296 --> 00:23:57.800 And it turns out that with simply ultra
low pass genome sequencing $150.00 a pop, you can focus on these different alterations that we identified and start to look. And in fact we were able to see in this example, this is a patient where we could identify the Richter’s alterations even close to five to six months before the actual diagnosis. So if you follow this in the blood, the blood cells have CLL at this time early on and it’s a very quiet genomic profile. Whereas the plasma shows all of these different alterations that match very similarly to what was
detected much later when the actual tissue diagnosis was made. We’ve been able to see that in a number of different other cases.

This is a nut. Whoopsie, this is another case. Well anyway, let’s see

where in the plasma we were able to again follow find those kind of Richter’s genomic alterations that was not evident in the blood cells. And finally, this is a case of a patient who went through transplant and we were able to identify post transplant
relapse months before the actual diagnosis

and then institute therapy and you

see those alterations go away again.

So I think just to summarize

this part of the talk,

I would say that we’ve been able to

actually find that the majority of

Richter’s does evolve from CLS subclones

through acquisition of additional drivers.

Clonally related Richter’s is

distinct from de Novo DLBCL.

There are molecular subtypes

of Richter’s that have and and

different subcategories do

have prognostic significance.

And then we’re very excited about
the self free DNA as a way to get us to non invasive earlier diagnosis because I think this could be really quite impactful for our patients. I think we’re always trying to. So I’m going to transition now in terms of talking about the immune microenvironment for Richter’s. You know, I think we’re always trying to gain a bird’s eye view of the landscape and really the advent of single cell analysis has really been so impactful all around. This is something I put together with
one of my postdoctoral fellows where we tried to look at across the field. You know single cell sequencing was named the method of the year in 2013 and then subsequently 2019 in multi omic analysis was the method of the year. CLL has had a bit of a lag time in terms of the the rest of the field, but again the easy access to material has really kind of stimulated us to start to look a little bit more closely. We’ve been able to apply this approach. Again I mentioned that Richter’s this area where the therapeutic opportunities are not great, but what has caught the attention of
00:27:07.972 --> 00:27:10.746 many is that it turns out that there is a response to immune checkpoint blockade.

00:27:10.746 --> 00:27:12.775 So fit 42 to 65% responses to PD1 blockade in Richter’s.

00:27:12.775 --> 00:27:16.062 This is really quite remarkable because a lot of blood B cell malignancies do not have a great response to PD anti PD one and so this sort of across these many studies.

00:27:18.280 --> 00:27:20.248 This raises the question are there determinants of response and resistance to PD1 blockade.

00:27:20.248 --> 00:27:22.549 We were able to partner together with our colleagues at MD Anderson.

00:27:22.549 --> 00:27:24.712 Again this is the work of Aaron.
Perry where they had already started

NOTE Confidence: 0.9313210728

a trial where they had patients

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initially on nivolumab and then then

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after the first cycle then ibrutinib

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was started and then response

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assessment happened at three months.

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And so we were able to collect bone

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marrow samples from these patients,

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a number in the green that had either

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a partial or complete response to

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patients that had progression even

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at the three month time point.

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And then just for comparison to CLL,

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patients were treated on the same

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trial and what Erin did was she was

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able to take marrow samples from

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these patients and through flow cytometry you can see that the small cells were the CLL cells, the large cells were the Richter’s and then there was another population here which was neither and this was the immune cells. And then she was able to perform a single cell characterization. And again to summarize a large body of work, what was really clear is that the responders compared to the non responders when you started to look
at all of those single cell transcriptomes, those there was a kind of a cluster of cells that kind of segregated with a unique phenotype and we called this cluster one. It turns out it was high expression for a transcriptional factor called Hobbit ZNF 683. And as she started to look at this population, she was able to perform some functional studies and demonstrate through cut and run and various over expression and knockout kind of analysis that ZNF 683 does appear to regulate T cell
pathways with activation cytotoxicity.

When we started to look at the trajectories the ZNF 683 high seemed to be a divergent pathway from terminal exhaustion. We also looked across other different solid tumor till settings and it turns out that the ZNF 683 high does mark a population that’s of patients that have better response to PD one therapy. Notably we looked at Melanoma across and other settings and also in she was also able to see that in the marrow but to make it more
clinically facile could could this be actually detected in the blood. And so she was able to look at independent patients who are responders or non responders on the MD Anderson trial. And in fact the responders have a very distinct profile in the blood T cells compared to the non responders where there is high expression of Z and F683 and and other cluster genes as well and this is we. So we were very proud of Aaron and Camila to get this into cancer cell. We actually tried to for a cover. It did not work. So you will never see this published.
only here in the seminar series. But we were trying to make a play on ZNF 683 and The Hobbit and the idea that if those of you were Middle Earth aficionados or token lovers, you know, the idea that you can either take a path and get to the valley of death with exhaustion or you can take a divergent pathway and end up back in the Shire happy. So that was our idea. Didn’t work. Whatever. So, so that. I’m going to move on to
the second set of study, second chapter shall we say in trying to look at function. And here you know in the same way that in the, in the genetic realm we’ve been able to study heterogeneity in patients. Well, can we not actually generate mice that are actually faithful to the disease through the by mimicking some of these genetic alterations that we’ve identified And then that provides us a platform with studying mechanism of disease and testing novel therapies.
And I just want to point out that there are different flavors of models. I don’t need to tell this audience or folks that yelled at, but the GEM models in general in, in particular I just want to point out have the advantage that this is kind of in a physiologic setting. It does allow us to look at tumor evolution and also immune micro environment analysis. And so for the past period of time, my group has really been interested in this question, well, how do you get from AB cell, what are the kind of pathway hits.
that happen that gets you to CLL? And can we study some of these alterations that we spent a lot of time genomically identifying such as SF3B1 or IK, CF3 or DMT3A and so and so forth and can we start to look at these things. So I won’t go over these past studies only to say that it has in fact been very gratifying to generate these mouse models and to demonstrate that, yes, these putative drivers that we’ve identified through sequencing actually generate CLL in mice. Most recently we had a very nice study ELISA 10 Hacken generated the setting where using CRISPR she
was able to introduce combinations of different alterations and release combinatorial study the different models of CLL and Richter’s that we identified. But for today, I’m going to talk about new unpublished data where we’ve been focused on one of the newer drivers that we identified, RPS 15 and some of the insights we’ve identified there. So RPS 15, what is it? It’s identified in 5% of patients. It’s enriched in patients who are relapsed following therapy.
It’s associated with shorter progression free survival and it commonly co-expresses with TP53.

One of the things that we found interesting about RPS15 is that there does seem to be a hotspot region where a lot of the alterations happen. And so this kind of piqued our interest in trying to learn more about RPS15. I do want to put this in the context that they’re across different cancers. There’s been a lot of different ribosomal mutations that have been found for CLLR. PS15 is the only ribosomal mutation that’s been identified. But certainly across other...
00:34:28.700 --> 00:34:30.280 cancers including breast cancer,
00:34:30.280 --> 00:34:31.784 Melanoma, myeloma,
00:34:31.784 --> 00:34:35.396 you see that this biology seems to be there.
00:34:35.400 --> 00:34:37.204 And carbosomopathies have been
00:34:37.204 --> 00:34:39.459 associated with a variety of
00:34:39.459 --> 00:34:41.160 different altered functions,
00:34:41.160 --> 00:34:44.580 so including DNA damage,
00:34:44.580 --> 00:34:46.900 proteasomal alteration and metabolic
00:34:46.900 --> 00:34:47.480 rewiring.
00:34:47.480 --> 00:34:50.352 So we were interested in trying to dig
00:34:50.352 --> 00:34:53.800 a little bit deeper about this in CLL.
00:34:53.800 --> 00:34:55.680 So we used our,
00:34:55.680 --> 00:34:57.568 we used this in a similar fashion to
00:34:57.568 --> 00:34:59.437 the other mice that we’ve generated.
00:34:59.440 --> 00:35:04.039 We introduced one of these hotspot mutations
00:35:04.040 --> 00:35:06.372
00:35:04.040 --> 00:35:06.798 that was intercross with CD19 cream mice.
NOTE Confidence: 0.808189956666667
00:35:06.800 --> 00:35:09.448 So this alteration is only present in B
NOTE Confidence: 0.808189956666667
00:35:09.448 --> 00:35:12.316 cells in the context of CD19 expression.
NOTE Confidence: 0.808189956666667
00:35:12.320 --> 00:35:15.833 So in B cells we were able to generate both
NOTE Confidence: 0.808189956666667
00:35:15.833 --> 00:35:18.198 heterozygous and homozygous mutated mice.
NOTE Confidence: 0.808189956666667
00:35:18.200 --> 00:35:21.714 We also intercross also with deletion 15,
NOTE Confidence: 0.808189956666667
00:35:21.720 --> 00:35:23.592 sorry TP 53,
NOTE Confidence: 0.808189956666667
00:35:23.592 --> 00:35:27.140 so that they were also mice that
NOTE Confidence: 0.808189956666667
00:35:27.140 --> 00:35:30.880 had double mutations as well.
NOTE Confidence: 0.808189956666667
00:35:30.880 --> 00:35:32.320 And so this is just a bit of
NOTE Confidence: 0.808189956666667
00:35:32.320 --> 00:35:33.120 the targeting strategy.
NOTE Confidence: 0.808189956666667
00:35:33.120 --> 00:35:34.866 This was really studies led by
NOTE Confidence: 0.808189956666667
00:35:34.866 --> 00:35:37.954 an MDPHD student and currently
NOTE Confidence: 0.808189956666667
00:35:37.954 --> 00:35:40.873 at MGH as a as an intern.
NOTE Confidence: 0.808189956666667
00:35:40.880 --> 00:35:43.666 And then Marwan Kwok is a awesome postdoc
NOTE Confidence: 0.808189956666667
00:35:43.666 --> 00:35:45.297 in my group right now who’s leading
up on some of the functional studies.
Neil Ruthin is in Graduate School in the New York area for computational biology.
So RPS 15 mutations, we’re very able through our mouse models to confirm that it does have oncogenic potential because certainly over time we’re able to identify that there is a population of RPS 15 mice that are do have expanded B cells. You can see this also in screen sizes over time. It does take quite a bit of time consistent with the human disease.
It does take about 15 months, about 818 months, 18 to 218 months to two years in order to see disease. So this is really a labor of love. But I would say that for sure with the RPS 15 mutations mutant mice we do see onset of disease less so with just the TP single mutant TP 53 but with a double mutant we also see not only CLL but evidence of Richter’s. But what was interesting is in the setting of hypo hyper proliferation when we look early on it seems to there seems to be hypoproliferation. So if we measure the B cell percentages
00:37:05.712 --> 00:37:07.862 in the homozygous mice in the setting of pre leukemia it’s actually depressed compared to wild type.

00:37:10.189 --> 00:37:12.199 So what is going on? How is this kind of hypoproliferation turning into hyper?

00:37:12.200 --> 00:37:13.640 So what is going on? How is this kind of hypoproliferation turning into hyper?

00:37:13.640 --> 00:37:16.622 And so to kind of gain some clues, we really focused on these pre leukemic mice for which we collected B cells and started off by just looking at gene expression profiling.

00:37:16.622 --> 00:37:18.113 And so to kind of gain some clues, we really focused on these pre leukemic mice for which we collected B cells and started off by just looking at gene expression profiling.

00:37:18.120 --> 00:37:20.000 And so to kind of gain some clues, we really focused on these pre leukemic mice for which we collected B cells and started off by just looking at gene expression profiling.

00:37:20.000 --> 00:37:22.328 And so to kind of gain some clues, we really focused on these pre leukemic mice for which we collected B cells and started off by just looking at gene expression profiling.

00:37:22.328 --> 00:37:24.410 And so to kind of gain some clues, we really focused on these pre leukemic mice for which we collected B cells and started off by just looking at gene expression profiling.

00:37:24.410 --> 00:37:27.024 And so to kind of gain some clues, we really focused on these pre leukemic mice for which we collected B cells and started off by just looking at gene expression profiling.

00:37:27.024 --> 00:37:29.154 And so to kind of gain some clues, we really focused on these pre leukemic mice for which we collected B cells and started off by just looking at gene expression profiling.

00:37:29.160 --> 00:37:31.120 And it became quite evident that there was quite a few different altered pathways including cell cycle checkpoints, MIC targets, DNA repair.

00:37:33.360 --> 00:37:35.600 And it became quite evident that there was quite a few different altered pathways including cell cycle checkpoints, MIC targets, DNA repair.

00:37:35.600 --> 00:37:37.624 And it became quite evident that there was quite a few different altered pathways including cell cycle checkpoints, MIC targets, DNA repair.
And looking close more closely, we could see that this was related to either reduction in proliferative capacity as well as there was increased G1 checkpoint activity after mitogenic stimulation and increased apoptosis. Now these alterations in cell cycle could be due to cell stress. So we started to look at the question of whether or not there was changes in oxidative stress and in fact using a Mitosox assay in our homozygous mice, we do see evidence both at baseline and with stimulation that there is increased enhanced oxidative stress which is supported by the.
00:38:22.126 --> 00:38:24.352 fact that when we use the inhibitor,

00:38:24.360 --> 00:38:27.076 so that pro oxidant we actually see

00:38:27.076 --> 00:38:30.285 that the RPS 15 mice are more sensitive

00:38:30.285 --> 00:38:33.878 to this inhibitor than the wild type.

00:38:33.880 --> 00:38:35.560 Now because of the cellular stress,

00:38:35.560 --> 00:38:39.040 does this actually can this actually

00:38:39.040 --> 00:38:44.144 support acquisition of genotoxic injury?

00:38:44.144 --> 00:38:45.730 And in this case,

00:38:45.730 --> 00:38:48.300 we were able to use gamma H2 AX and

00:38:48.300 --> 00:38:50.421 we see in the homozygous mice that

00:38:50.421 --> 00:38:52.593 there is increase in gamma H2AX.

00:38:52.593 --> 00:38:55.158 And as we started to,

00:38:55.160 --> 00:38:56.636 there’s a lot of westerns that

00:38:56.636 --> 00:38:57.880 I could have shown you.

00:38:57.880 --> 00:39:00.575 But suffice it to say that through
examination of the mutant mice,

we do see impaired cell cycle checkpoint response to DNA damage,

impaired response signaling,

abrogation of ATM and CHECK 2 signaling and heightened intrinsic aberrant DNA damage response.

And Despite that, there’s also increased proliferation signaling.

So one of our highest hits in our gene expression was ZAP 70, which has relevance to CLL.

So we see that here.

And there’s also enhanced ABCR signaling.

So definitely a balance between different forces at play.
Going on, our next question was that is seeing these different sort of phenotypes, since this is a ribosomal protein, is there actually alteration? Is there effects of mutant RPS 15 on translation? And so we asked could RPS 15 mutation cause ribosomes to preferentially translate certain genes? Could the mutation cause ribosomes for example to stall at specific protein coding sequence motifs interrupting translation of certain genes or could it read through stop codons and then result in
misfolded and degraded proteins?

And so for this we performed A ribosomal profiling.

And when we started to look at whether or not there was evidence of differential translation efficiency, there were certainly many genes that were appeared to be have enhanced or depressed translational efficiency.

And as we started to look at the pathways that were impacted, these included many of those pathways that I already talked to you about in the pre leukemic setting.

So cell cycle, MC target, cell cycle checkpoints and DNA replication.
And specific examples that we could see were genes that have very well known roles in these different pathways. We were able to support this kind of ribosome riboseek analysis by looking at protein expression. So that was actually very nice to see that linkages between translation and the pathways that we were impacting.
When we started to look at, we were also able to see evidence not only in murine cells but also in a human cell line. We saw evidence of stop codon stalling. So you can see kind of a pile up here in terms of the relative position to the stop codon, but we also saw evidence of stop codon read through. And so we do see that there's enrichment of certain codons in that kind of stop site, suggesting that this is not a random process, but there's actually motifs that are kind of guiding this process.
00:42:20.624 --> 00:42:23.088 as we started to look at the

leukemic B cells,

00:42:24.080 --> 00:42:28.200 we could see up regulation of Mick targets.

00:42:29.800 --> 00:42:31.354 but only to say that as we

start to go through our model

of what we think is going on,

00:42:32.911 --> 00:42:34.836 of what we think is going on,

00:42:34.840 --> 00:42:38.438 we do see that in this mutated

ribosomal protein that there is

evidence of altered translation

00:42:40.785 --> 00:42:42.661 evidence of altered translation

00:42:42.661 --> 00:42:44.809 through a couple of different

mechanisms that these do initially

lead to hypoproliferation.

00:42:48.680 --> 00:42:53.800 There is elevated ZAP 70 and BCR

00:42:53.800 --> 00:42:55.760 signaling as well as make activation.
But in initially there’s P53 mediated apoptosis and cell cycle checkpoint changes that are leading to that hyper proliferation, but that over time there’s acquisition of DNA damage and genomic instability that tip the balance and get us to the state of hyper proliferation.

So just to conclude this part of the talk, I’ll just say that again our new work suggests that RPS 15 mutation is ACL driver and reinforces the notion that CLL has these core pathways that are affected. So I didn’t go into this, but across our different mouse models...
we are seeing common pathways through different mechanisms that appear to be involved and current ongoing work is starting to look at the immune micro environment so that we can start to link the genotype with whether or not they’re related to distinct changes in the immune microenvironment.

In the final slides, I just want to say that you know I think across the field has been really focused on the blood easy access,
lots of tumor there.

But I think increasingly we do need to look at these specialized hematolymphoid organs where there is a specialized immune microenvironment that we can understand better.

I think that there is a priority and interest in trying to go earlier in disease.

So how can we understand those early events? How can we intervene early? How can we change Natural History? We're only going to get there by understanding a little bit more about this earlier time Multiomic profiling for sure.

There's so much data out there and how
00:44:50.111 --> 00:44:52.327 can we link them all together and kind

00:44:52.390 --> 00:44:54.679 of not have them as separate entities,

00:44:54.680 --> 00:44:57.350 but really trying to coalesce

00:44:57.350 --> 00:45:00.636 into kind of archetypes that we

00:45:00.636 --> 00:45:02.562 can understand spatial analysis.

00:45:02.562 --> 00:45:05.208 So our group is actively working

00:45:05.208 --> 00:45:08.301 on efforts to try to look at the

00:45:08.301 --> 00:45:10.155 architecture of lymph nodes and

00:45:10.155 --> 00:45:14.068 bone marrow to see how malignant

00:45:14.068 --> 00:45:16.636 cells are organized and also in

00:45:16.636 --> 00:45:18.600 relationship to their genotype.

00:45:18.600 --> 00:45:22.104 So their mutations and do specific

00:45:22.104 --> 00:45:25.240 clones segregate with specific types

00:45:25.240 --> 00:45:29.490 of niches and are they organized

00:45:29.490 --> 00:45:31.240 in certain type of neighborhoods.
And finally I touched upon with our self free DNA work some of the early detection. I’m going to end with the last couple slides speaking about early intervention we hope in the future. But another big part of the work that my group does is think about cancer neo antigens. And from all the genomic studies that we’ve been doing, we’ve realized that there’s the opportunity for these mutations to generate epitopes that can be recognized by T cells. I’m not going to go into this in great length only to say that there’s
straightforward algorithms by now that allow us to take start with the sequencing data and identify for us what those new antigens might be. I want to say that some of our earliest work in the new antigen field and kind of setting up these pipelines were in CLL because that is where we had the data and all the tools to help us construct some of the these first pipelines that were available. And certainly our vaccine neo antigen work that Doctor Weiner alluded to has taken our group very far afield from CLL. We’ve gone into the solid tumors and
we’ve been able to conduct some early proof of concept studies that such an approach of starting with tumor looking for genomic alterations and generating a personal vaccine is feasible. But I’ve always been super interested in trying to bring it back to CLL. And so I’m happy to say that right now we have a phase one study for patients with unmutated IGHV led by and supported by Matt Davids and Jennifer Brown to study and look at the impact of this vaccine alone vaccine together with low dose cyclophosphamide as a way to kind of alter the immune micro environment.
and maybe address T regs.

And then also a third cohort to actually add immune checkpoint blockade together and

we already have enrolled in a number the first three patients we’re already seeing interesting immune responses compared to our solid tumor settings.

These are patients who actively have circulating disease.

So is it possible to even vaccinate and generate meaningful responses when there’s leukemia that’s in circulation?

And the short answer, it seems like yes.

So we’re we are actually seeing
very nice brisk immune responses to actually some of our patients. So I hope you stay tuned and hopefully we’ll have more to say about that in the time to come. I’ve tried to acknowledge folks along the way, but here’s a more extensive list and I really appreciate your attention and thank you. Yes. So how do you think about driver mutations, specific driver mutations related to transformation, related to potential for these differentiation B cells
leading to the clinical outcome?

You listed a whole list of potential driver mutations. It’s not clearly what the individual driver mutations are doing. And how you think about getting the answer to that question, if it is an important question, yeah, no, I think I skipped over a lot of stuff. And so I think one of the things that we can do when we have these driver lists because we can see whether they segregate into particular pathways. And by virtue of kind of separating
out the CLL versus Richter clones,

we were able to kind of identify which

of those drivers seem to be CLL,

which were Richter’s and which were which

were in a path on the way to transformation.

And so some of those pathways that

we see affected are related to Mick,

for example, they’re related to cell cycles.

So this it’s not a surprise,

but it and metabolic rewiring as well.

So there’s many.

So I think the drivers do help us think

about the biology of what is going on,

but I hope that we can also

use them as ways to for early detection.

I don’t know if this is answers
your question,

but

yeah, I don’t want the questions online,

but what what do you think about the role of RGS 15 in normal CD5B cells?

So it’s there, yes.

So the question is what is its function in thinking about what CD5B cells are doing in terms of maintenance and tolerance for example and their potential product activity and where they are,

right. So we haven’t looked into that.

I mean I think we have the tools and

so we’ve we’ve really been focused on the,
the mutant setting. Yeah.

But I think it’s a really interesting question and I think that it would be a separate question where it could be like all of these different mutations that we’re finding. Yes.

Yes the genes and what are their roles in normal business.

I think I think you are absolutely correct. Yeah. Yes.

You said that the unmutated CLLS have a re urgent headed with the nursery you said that the unmutated have a far longer list of mutated drivers in unmutated
00:51:39.080 --> 00:51:41.816 CLI see. So I guess the question then
00:51:41.816 --> 00:51:44.638 is do you think that the mechanism
00:51:44.638 --> 00:51:48.110 that’s leading to the mutations of the
00:51:48.110 --> 00:51:51.512 IGH locus is unrelated to the genetic
00:51:51.512 --> 00:51:53.696 diversity that we’re getting or is there
00:51:53.696 --> 00:51:55.720 a relations to them and how does that I
00:51:55.720 --> 00:51:57.078 I I think that’s a great question. 
00:51:57.080 --> 00:51:58.920 So the the question is whether or not
00:52:00.960 --> 00:52:06.640 how the immunoglobulin mutational status
00:52:06.640 --> 00:52:10.518 relates to kind of the genetic diversity.
00:52:10.520 --> 00:52:13.160 So. So yeah, it’s been understood
00:52:13.160 --> 00:52:16.926 that whether or not the CL LS have a
00:52:16.926 --> 00:52:18.645 mutated or unmutated immunoglobulin
00:52:18.645 --> 00:52:22.035 relates to their cell of origin,
00:52:22.040 --> 00:52:25.152 kind of where are they in kind of B
cell development and whether or not those kind of normal physiological mutational processes are are present. So I think it does speak to the underlying biology of that cell and probably it helps us understand why there there could be more mutations in these different genes compared to the unmutated. So Marcus Bosenberg asks are there any recurrent genetic or epigenetic changes in CLL arising at later time points in RPS 15?
Marcus, hello, great question.

We haven’t actually looked at that.

I think that’s a great question and probably something I should take back to the group and we should look, but we haven’t.

So thank you.

One last question, there’s George Miller asks, can you comment on the role of Epstein Barr virus in conversion of CLL to PLVCL?

I really can’t maybe yes, we have not looked at that.
It’s a great question and certainly EBB does play a role in immortalization of B cell lines. But I don’t have much deep thoughts about that. So my regrets. Thank you. Well, thank you very much for visiting us. Yes, thank you.