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 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 W u here. She was a fellow at Dana Farber and of...
00:01:26.924 --> 00:01:30.856 course it’s still with Dana Farber when
00:01:30.856 --> 00:01:35.056 I was a substantially younger attending.
00:01:35.056 --> 00:01:39.440 And in fact we worked together in clinic,
00:01:40.278 --> 00:01:43.630 yes, briefly.
00:01:43.720 --> 00:01:47.160 She dabbled a little bit in seeing a
00:01:47.160 --> 00:01:50.520 patient with breast cancer or one or two.
00:01:50.520 --> 00:01:53.805 And so I’ve known Kathy now for 20
00:01:53.805 --> 00:01:57.480 plus years and Kathy has built really
00:01:57.480 --> 00:01:59.960 a phenomenal career at Dana Farber.
00:01:59.960 --> 00:02:01.600 Her own interests are broad.
00:02:01.600 --> 00:02:02.912 I didn’t know before,
00:02:02.920 --> 00:02:05.349 which is that she even had an
00:02:05.349 --> 00:02:07.898 interest in sickle cell disease and
00:02:07.898 --> 00:02:09.806 therapeutic approaches to sickle
00:02:09.806 --> 00:02:12.040 cell disease way back when,
NOTE Confidence: 0.8770731
00:02:12.040 --> 00:02:14.070 but ultimately decided that some
NOTE Confidence: 0.8770731
00:02:14.070 --> 00:02:16.680 some amount of focus was needed.
NOTE Confidence: 0.8770731
00:02:16.680 --> 00:02:18.960 And her interests have really
NOTE Confidence: 0.8770731
00:02:18.960 --> 00:02:21.240 focused on immunotherapy and Col.
NOTE Confidence: 0.8770731
00:02:21.240 --> 00:02:23.836 and and beyond that,
NOTE Confidence: 0.8770731
00:02:23.836 --> 00:02:27.081 the development of vaccines and
NOTE Confidence: 0.8770731
00:02:27.081 --> 00:02:30.639 and tumor specific vaccines.
NOTE Confidence: 0.8770731
00:02:30.640 --> 00:02:33.040 She is presently a Professor of
NOTE Confidence: 0.8770731
00:02:33.040 --> 00:02:35.120 Medicine at Harvard Medical School
NOTE Confidence: 0.8770731
00:02:35.120 --> 00:02:38.480 and the chief of the Division of
NOTE Confidence: 0.8770731
00:02:38.480 --> 00:02:40.160 Let Me See If I Get This Right,
NOTE Confidence: 0.8770731
00:02:40.160 --> 00:02:42.092 Stem cell transplantation and
NOTE Confidence: 0.8770731
00:02:42.092 --> 00:02:44.507 cellular therapies at the the
NOTE Confidence: 0.8770731
00:02:44.507 --> 00:02:46.580 Dana Farber Cancer Institute.
NOTE Confidence: 0.8770731
00:02:46.580 --> 00:02:51.400 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.

We had I will, 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 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 and 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,
00:04:56.280 --> 00:04:57.320 for better or for worse.
NOTE Confidence: 0.76337852
00:04:57.320 --> 00:04:57.650 Unfortunately,
NOTE Confidence: 0.76337852
00:04:57.650 --> 00:04:59.960 by the time that we are diagnosing
NOTE Confidence: 0.76337852
00:04:59.960 --> 00:05:01.040 patients with cancer,
NOTE Confidence: 0.76337852
00:05:01.040 --> 00:05:03.301 we’re really here at the time of
NOTE Confidence: 0.76337852
00:05:03.301 --> 00:05:05.600 escape where there’s already so many
NOTE Confidence: 0.76337852
00:05:05.600 --> 00:05:06.863 different resistance mechanisms
NOTE Confidence: 0.76337852
00:05:06.863 --> 00:05:09.183 that have really come into play
NOTE Confidence: 0.76337852
00:05:09.183 --> 00:05:11.185 that make the tumor fit to expand
NOTE Confidence: 0.76337852
00:05:11.185 --> 00:05:14.120 and grow in the patient host.
NOTE Confidence: 0.76337852
00:05:14.120 --> 00:05:15.405 We also increasingly know that
NOTE Confidence: 0.76337852
00:05:15.405 --> 00:05:17.159 this is not happening in a vacuum,
NOTE Confidence: 0.76337852
00:05:17.160 --> 00:05:18.492 that there’s an interaction
NOTE Confidence: 0.76337852
00:05:18.492 --> 00:05:20.157 with the host immune system.
NOTE Confidence: 0.76337852
00:05:20.160 --> 00:05:20.666 But again,
NOTE Confidence: 0.76337852
00:05:20.666 --> 00:05:22.437 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, but also how do we understand this, these heterogeneous 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 be readily
NOTE Confidence: 0.76337852
00:06:21.365 --> 00:06:23.235 accessible directly from the patients.
NOTE Confidence: 0.76337852
00:06:23.240 --> 00:06:24.997 The other thing is for a cancer,
NOTE Confidence: 0.76337852
00:06:25.000 --> 00:06:26.488 it’s quite indolent.
NOTE Confidence: 0.76337852
00:06:26.488 --> 00:06:28.910 And So what that means is that
NOTE Confidence: 0.76337852
00:06:28.910 --> 00:06:30.340 we really have really long
NOTE Confidence: 0.76337852
00:06:30.401 --> 00:06:32.117 disease histories of patients.
NOTE Confidence: 0.76337852
00:06:32.120 --> 00:06:35.018 We can really take snapshots in
NOTE Confidence: 0.76337852
00:06:35.018 --> 00:06:37.733 time and study evolution in real
NOTE Confidence: 0.76337852
00:06:37.733 --> 00:06:39.552 time along with the patient.
NOTE Confidence: 0.76337852
00:06:39.552 --> 00:06:41.760 And so for some time now,
NOTE Confidence: 0.76337852
00:06:41.760 --> 00:06:44.316 our group together with colleagues
NOTE Confidence: 0.76337852
00:06:44.316 --> 00:06:47.457 in the Boston area,
NOTE Confidence: 0.76337852
00:06:47.457 --> 00:06:49.080 we’ve actually had this program
NOTE Confidence: 0.76337852
00:06:49.080 --> 00:06:49.924 where we’ve been trying to study
NOTE Confidence: 0.76337852
00:06:49.924 --> 00:06:49.981 the link from genome to phenome.
NOTE Confidence: 0.76337852
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 and next in terms of the genomics. Again, as I said this is a very in general for cancer an
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 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. 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, and then 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 puts 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 studies were about 100 patients and then around 2015 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 time frame that I’m speaking there has been the introduction of targeted inhibitors of BCL,
two 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
immitating commute plot, but I think it just speaks of a number of different things. First, I want to acknowledge the young people who were the leaders of this project. It was really an international collaboration. So I had the pleasure of working with Binyamin Nisbacher and Ziao Lin and Gaddy Goetz’s group computational gurus and then Cindy Hahn from Dana Farber. Awesome lymphoma oriented fellow and then Ferran, Nadeau and Marty from the group in Spain, the Spanish CLL group in 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, 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 get 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 more 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 unmutated.

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.

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. And what was super interesting is 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
00:15:17.874 --> 00:15:19.118 final data slide related to this,
00:15:19.120 --> 00:15:21.535 which is indeed when we kind of
00:15:21.535 --> 00:15:23.504 breakdown the samples based on
00:15:23.504 --> 00:15:25.240 their classical clinical group,
00:15:25.240 --> 00:15:26.760 based on the expression clusters,
00:15:26.760 --> 00:15:29.225 whether they were concordant or
00:15:29.225 --> 00:15:31.197 discordant to that classification,
00:15:31.200 --> 00:15:33.984 we could actually see differences in
00:15:33.984 --> 00:15:35.840 their clinical outcomes suggesting
00:15:35.907 --> 00:15:38.157 that our expression cluster system
00:15:38.157 --> 00:15:40.280 was actually increasing the accuracy
00:15:40.280 --> 00:15:42.520 of what we’re trying to do in
00:15:42.520 --> 00:15:44.320 terms of prognostication.
00:15:44.320 --> 00:15:46.240 So we’ve been really excited to,
00:15:46.240 --> 00:15:47.440 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 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, the majority are clonally unrelated. We know that because if we follow their 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. I'm so sorry. Please get your affairs and orders that there's really not
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 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 this has 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 or Gem models to try to understand this. And there’s been actually a lot of progress in understanding the genome looking at the
epigenetics and the transcriptomics.

And So what I’m going to demonstrate is some of our efforts in this area. This is really work that’s been that was 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. So in other words, not just Richter samples in isolation.
but antecedent CLL matched together

This was about 50 patients

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

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 As I said the artifact from FFPE.

00:21:02.536 --> 00:21:05.112 And then we were able to put in our

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 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 then using those data we performed unbiased NMF clustering.
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, so this is clonally unrelated Richter. So 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
00:22:37.943 --> 00:22:41.055 actually it appears to be molecular
NOTE Confidence: 0.912607754827586
00:22:41.055 --> 00:22:43.520 subtypes within Richter’s itself
NOTE Confidence: 0.912607754827586
00:22:43.520 --> 00:22:47.205 and these TP 53 has long been
NOTE Confidence: 0.912607754827586
00:22:47.205 --> 00:22:49.060 associated with Richter’s but we can
NOTE Confidence: 0.912607754827586
00:22:49.060 --> 00:22:50.235 see that there’s different flavors.
NOTE Confidence: 0.912607754827586
00:22:50.240 --> 00:22:54.048 So this one here has enrichment in
NOTE Confidence: 0.912607754827586
00:22:54.048 --> 00:22:56.784 whole genome doubling this group.
NOTE Confidence: 0.912607754827586
00:22:56.784 --> 00:23:00.096 Here RS3 has Co occurrence with
NOTE Confidence: 0.912607754827586
00:23:00.096 --> 00:23:04.140 Notch one also deletion 15 Q which
NOTE Confidence: 0.912607754827586
00:23:04.140 --> 00:23:08.972 covers MGA which is effects Mick
NOTE Confidence: 0.912607754827586
00:23:08.972 --> 00:23:13.135 and then RS5 also has Notch one
NOTE Confidence: 0.912607754827586
00:23:13.135 --> 00:23:16.554 as well wild type Notch one and a
NOTE Confidence: 0.912607754827586
00:23:16.554 --> 00:23:18.239 lot of copy number alterations.
NOTE Confidence: 0.912607754827586
00:23:18.240 --> 00:23:19.460 There were also two other
NOTE Confidence: 0.912607754827586
00:23:19.460 --> 00:23:20.680 subtypes that did not have
NOTE Confidence: 0.76865602
00:23:22.720 --> 00:23:25.656 TP53K Ras S Pen, Notch one together with
Trisomy 12 and also SF3B1 with EGR Two.
And again these different subgroups appear to have different clinical behavior where the ones that have TP 53 seem to have worse prognosis. Now what is the meaning of kind trying to look at all these different genomic alterations? Well one thing we realized is that maybe we could harness all of this and actually look to see this, whether this could help us devise a non-invasive approach to identifying Richter’s and getting us to earlier detection. 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, very quiet genomic profile. Whereas the plasma shows all of these different alterations that match very similarly to what was
00:24:38.691 --> 00:24:41.038 detected much later when the actual
00:24:41.040 --> 00:24:43.398 the the tissue diagnosis was made.
00:24:43.400 --> 00:24:45.400 We’ve been able to see that in a
00:24:45.400 --> 00:24:47.278 number of different other cases.
00:24:47.280 --> 00:24:48.080 This is a nut.
00:24:48.080 --> 00:24:51.000 Whoopsie, this is another case.
00:24:51.000 --> 00:24:51.760 Well anyway,
00:24:51.760 --> 00:24:52.520 let’s see
00:24:54.640 --> 00:24:59.920 where the in the plasma we were able
00:24:59.920 --> 00:25:02.719 to again follow find those kind of
00:25:02.720 --> 00:25:04.192 Richter’s genomic alterations that
00:25:04.192 --> 00:25:08.320 was not evident in the blood cells.
00:25:08.320 --> 00:25:10.464 And finally, this is a case of a
00:25:10.464 --> 00:25:12.718 patient who went through transplant and
00:25:15.200 --> 00:25:18.175 we were able to identify post transplant

43
relapse months before the actual diagnosis
and then institute therapy and you
see those alterations go away again.
So I think just to summarize
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 these 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. 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 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 area where the therapeutic opportunities are not great, but what has caught the attention of
many is that it turns out that there is a response to immune checkpoint blockade. So fit 42 to 65% responses to PD1 blockade in Richter’s. 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. This raises the question are there determinants of response and resistance to PD1 blockade. We were able to partner together with our colleagues at MD Anderson. Again this is the work of Aaron.
Perry where they had already started

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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
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 that were in the bone marrow. 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 different sorts of over expression and knockout kind of analysis that ZNF 683 does appear to regulate T cell
00:29:23.656 --> 00:29:26.400 pathways with activation cytotoxicity.

00:29:26.400 --> 00:29:28.542 When we started to look at the trajectories the ZNF 683 high seemed to be a divergent pathway from terminal exhaustion.

00:29:30.249 --> 00:29:32.427 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.

00:29:34.320 --> 00:29:37.278 Notably we looked at Melanoma across and other settings and also in she was also able to see that you know we did our analysis 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.
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
NOTE Confidence: 0.808189956666667
00:33:14.540 --> 00:33:16.435 was able to introduce combinations
NOTE Confidence: 0.808189956666667
00:33:16.435 --> 00:33:18.980 of different alterations and release
NOTE Confidence: 0.808189956666667
00:33:18.980 --> 00:33:21.032 combinatorial study the different models
NOTE Confidence: 0.808189956666667
00:33:21.032 --> 00:33:23.440 of CLL and Richter’s that we identified.
NOTE Confidence: 0.808189956666667
00:33:23.440 --> 00:33:25.407 But for today, I'm going to talk
NOTE Confidence: 0.808189956666667
00:33:25.407 --> 00:33:26.817 about new unpublished data where
NOTE Confidence: 0.808189956666667
00:33:26.817 --> 00:33:28.602 we've been focused on one of the
NOTE Confidence: 0.808189956666667
00:33:28.602 --> 00:33:30.317 newer drivers that we identified,
NOTE Confidence: 0.808189956666667
00:33:30.320 --> 00:33:33.113 RPS 15 and some of the insights
NOTE Confidence: 0.808189956666667
00:33:33.113 --> 00:33:34.840 that we've identified there.
NOTE Confidence: 0.808189956666667
00:33:34.840 --> 00:33:38.200 So RPS 15, what is it?
NOTE Confidence: 0.808189956666667
00:33:38.200 --> 00:33:41.572 It is a ribosomal protein.
NOTE Confidence: 0.808189956666667
00:33:41.572 --> 00:33:45.099 It’s identified in 5% of CLL patients.
NOTE Confidence: 0.808189956666667
00:33:45.099 --> 00:33:47.864 It’s enriched in patients who
NOTE Confidence: 0.808189956666667
00:33:47.864 --> 00:33:50.759 are relapsed following therapy.
NOTE Confidence: 0.808189956666667
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
cancers including breast cancer,

Melanoma, myeloma,
you see that this biology seems to be there.

And carbosomopathies have been associated with a variety of different altered functions,

so including DNA damage,

proteasomal alteration and metabolic rewiring.

So we were interested in trying to dig a little bit deeper about this in CLL.

So we used our, we used this in a similar fashion to the other mice that we’ve generated.

We introduced one of these hotspot mutations

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that was intercross with CD19 cream mice.

So this alteration is only present in B cells in the context of CD19 expression.

So in B cells we were able to generate both heterozygous and homozygous mutated mice.

We also intercross also with deletion 15, sorry TP 53, so that they were also mice that had double mutations as well.

And so this is just a bit of the targeting strategy.

This was really studies led by an MDPHD student and currently at MGH as an intern.

And then Marwan Kwok is an awesome postdoc 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 to 218 months 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
in the homozygous mice in the setting of pre leukemia it’s actually depressed compared to wild type. So what is going on? How is this kind of hypoproliferation turning into hyper? 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. And it became quite evident that there was quite a few different altered pathways including cell cycle checkpoints, MIC targets, DNA repair.
And looking 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
fact that when we use the inhibitor, so that pro oxidant we actually see that the RPS 15 mice are more sensitive to this inhibitor than the wild type. Now because of the cellular stress, does this actually can this actually support acquisition of genotoxic injury? And in this case, we were able to use gamma H2 AX and we see in the homozygous mice that there is increase in gamma H2AX. And as we started to, there’s a lot of westerns that I could have shown you. 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 appeared to 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 analysis by looking at protein expression and we can confirm that what we saw as having depressed translation. So the GPX one we could actually confirm at the protein level for GPX 1 and O2O2 four and increase expression in PTP 4A2. 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.
as we started to look at the leukemic B cells, we could see up regulation of Mic targets.
And I’m going to just skip over this, but only to say that as we start to go through our model of what we think is going on, we do see that in this mutated ribosomal protein that there is evidence of altered translation through a couple of different mechanisms that these do initially lead to hypoproliferation. There is elevated ZAP 70 and BCR 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 micro environment.

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
can we link them all together and kind of not have them as separate entities, but really trying to coalesce into kind of archetypes that we can understand spatial analysis. So our group is actively working on efforts to try to look at the architecture of lymph nodes and bone marrow to see how malignant cells are organized and also in relationship to their genotype. So their mutations and do specific clones segregate with specific types of niches and are they organized 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 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 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, specific driver mutations 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
00:50:12.520 --> 00:50:12.920 your question,
00:50:12.920 --> 00:50:13.160 but
00:50:15.200 --> 00:50:16.960 yeah, I don’t want the questions online,
00:50:16.960 --> 00:50:19.360 but what what do you think about the
00:50:19.360 --> 00:50:23.220 role of RGS 15 in normal CD5B cells?
00:50:23.220 --> 00:50:25.280 So it’s there, yes.
00:50:25.280 --> 00:50:27.583 So the question is what is its
00:50:27.583 --> 00:50:29.279 function in thinking about what
00:50:29.280 --> 00:50:32.121 CD5B cells are doing in terms of
00:50:32.121 --> 00:50:33.189 maintenance and tolerance for
00:50:33.189 --> 00:50:34.631 example and their potential product
00:50:34.631 --> 00:50:36.116 activity and where they are,
00:50:36.320 --> 00:50:38.315 right. So we haven’t looked into that.
00:50:38.320 --> 00:50:43.600 I mean I think we have the tools and
00:50:43.600 --> 00:50:47.298 so we’ve we’ve really been focused on the,
00:50:47.298 --> 00:50:48.825 the mutant setting. Yeah.
NOTE Confidence: 0.911263191428571

00:50:48.825 --> 00:50:53.080 But I I think it’s a really interesting
NOTE Confidence: 0.911263191428571

00:50:53.080 --> 00:50:57.960 question and I think that it would
NOTE Confidence: 0.911263191428571

00:50:57.960 --> 00:50:59.795 be a separate question where it
NOTE Confidence: 0.911263191428571

00:50:59.795 --> 00:51:01.839 could be like all of these different
NOTE Confidence: 0.803011412

00:51:04.160 --> 00:51:06.560 mutations that we’re finding. Yes.
NOTE Confidence: 0.803011412

00:51:06.560 --> 00:51:08.480 Yes the the genes and and what are
NOTE Confidence: 0.803011412

00:51:08.480 --> 00:51:09.960 their roles in in normal business.
NOTE Confidence: 0.34478727

00:51:12.320 --> 00:51:14.754 I think I I I think you are
NOTE Confidence: 0.34478727

NOTE Confidence: 0.34478727

00:51:17.920 --> 00:51:21.210 Yeah I’m I’m getting discredited I think
NOTE Confidence: 0.34478727

00:51:21.210 --> 00:51:23.586 you said that the unmutated CLLS have
NOTE Confidence: 0.34478727

00:51:23.586 --> 00:51:25.637 a re urgent headed with the nursery
NOTE Confidence: 0.775221385555556

00:51:26.680 --> 00:51:30.624 so yeah so the quest so the unmutated
NOTE Confidence: 0.775221385555556

00:51:30.624 --> 00:51:34.444 has there are there’s a far longer
NOTE Confidence: 0.775221385555556

00:51:34.444 --> 00:51:38.000 list of mutated drivers in unmutated

84
So I guess the question then is do you think that the mechanism that’s leading to the mutations of the IGH locus is unrelated to the genetic diversity that we’re getting or is there a relation to them and how does that I think that’s a great question. So the question is whether or not how the immunoglobulin mutational status relates to kind of the genetic diversity. So. So yeah, it’s been understood that whether or not the CL LS have a mutated or unmutated immunoglobulin relates to their cell of origin, 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 of origin and probably it helps us understand why there there could be more mutations in 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.