CLL Global's Patient-Focused Research Symposium
July 2026

Steve Winfield:
Hello. I'm Steve Winfield, chairman of the board of the CLL Global Research Foundation. Thank you for joining our patient-focused research symposium. I'd like to take a moment to share why your support is so important. CLL Global's mission is not only to find a cure for CLL, but also to help patients live longer, healthier lives. Every project we fund is focused on improving every life that is touched by CLL.

In today's world, the need for research funding is greater than ever, and CLL Global must step in to support critical research and the brilliant young investigators driving it forward. More than 95 percent of every dollar donated to CLL Global goes directly to research. Your support funds the innovative ideas and breakthroughs you'll hear about today.

If you've donated in the past, thank you. You have helped make this progress possible. If you're able, I encourage you to consider donating today. Together, we can continue advancing research to bring us closer to better treatments, and, ultimately, to a cure.

Our last symposium focused on MD Anderson researchers. Today, we highlight global researchers from top institutions in Australia, Massachusetts, and Ohio. Thank you for your support. I hope you enjoy the symposium.

Dr. Jennifer Woyach: Hi, everybody. My name is Jennifer Woyach, from the Ohio State University, and I'm excited to talk to you today about our research looking at whole genome doubling, or tetraploidy, in chronic lymphocytic leukemia and Richter's transformation.

Richter's transformation is a transformation of CLL to a more aggressive lymphoma. And although we think about this transformation as being relatively sudden, it's more of a spectrum where the CLL cells start to take on characteristics that make them behave less like typical CLL, and more like an aggressive lymphoma, with more lymph node involvement, more whole-body or constitutional symptoms. And this is associated with a number of genetic abnormalities, which you can see here. And overall, they, in general, make the cells more genetically unstable.

About 15 percent of Richter's transformation is associated with a specific gene alteration that's called tetraploidy, which is where all of the chromosomes get doubled. This is often associated with other high-risk gene mutations like TP53 mutations. And we found a number of years ago that tetraploidy in CLL cells, so if the CLL cells show doubling of the chromosomes, that's associated with a high risk of Richter's transformation in patients who are undergoing treatment with ibrutinib (Imbruvica).

Tetraploidy is something that occurs in many types of cancers, both in blood cancers, as well as solid tumors, and it makes the cells more easily able to acquire additional gene abnormalities like mutations or other chromosome changes. And the goal of our research is to understand the basis and the consequences caused by tetraploidy in CLL, and how this can cause or help facilitate transformation to Richter's. Our hypothesis is that the tetraploid clone that we can find in CLL is actually a precursor to Richter's transformation, and that it has unique genetic and other features that we typically associate with Richter's.

This is some work that was done by Anthony Mansour, who was a resident with us at Ohio State, and is currently a heme/onc Fellow at City of Hope. We looked at about 80 patients that were treated at the Ohio State University, who were shown to have tetraploidy on chromosome analysis. And we found that over time, actually, about half of them developed Richter's transformation. And that the patients who developed Richter's transformation, it tended to be associated more commonly with other mutations like TP53 mutations, deletions of a gene called CDKN2A or N2B, which are also associated with Richter's.

In order to study tetraploid in the laboratory, we made cell lines tetraploid by treating them with chemicals that interrupt the cell cycle, and we used two well-characterized CLL cell lines called OSU-CLL and MEK-1. And then, we separate out the tetraploid cells from what are called diploid cells, which are normal copies of the chromosomes. And all the lab work I'm going to show you has been done by Samon Benrashid, who is a graduate student who works with me.

These are called Circos plots, and they're used to show changes in the genome. And we did a procedure here that's called optical genome mapping, where we unwind the DNA of cells, and then fluorescent probes are attached that can tell whether specific parts of the DNA are normal or abnormal. And then, a special microscope is used to capture those fluorescent probes, to tell where and what type of changes have occurred. So, all the numbers that are on these circles correspond to different chromosomes, and each color within them is a different type of genetic abnormality.

Here, we're comparing a cell line OSU-CLL that is normal wild type, normal number of chromosomes, to ones that we made tetraploid. And you can see pretty clearly that the cells that are tetraploid start off with more genetic changes, in addition to just having the doubling of the chromosomes. Then, we took the cell lines and grew them in culture until they divided 20 times, and looked at the genome again, and we saw that the tetraploid cells acquire additional abnormalities more quickly than those that have normal amounts of chromosomes.

We then looked at cells from CLL patients with tetraploid, where we collected samples over time, and we separated out the tetraploid cells and did this optical genome analysis

again. And you can see, compared to the sample from 2008, a couple years later, you can see a lot more genetic changes.

To understand these specific genetic changes that were occurring, we took samples from patients with tetraploidy, where we had samples collected over time. We separated out the tetraploid cells from the normal diploid cells, and then we performed RNA sequencing of each of those different compartments at the different time points. And this is just a representative sample from a patient where we had cells stored in 2011, when tetraploidy was first noted, and 2013, when the patient developed Richter's transformation.

When we look at specifically the early time point, here, we're looking at the normal diploid cells compared to the tetraploid cells, and we see that there's actually a number of differences between the normal chromosome cells and those with double chromosomes. We see a lot of expression of genes involved in inflammatory responses that we often associate with Richter's transformation.

Now, we're looking at our later time point. And we, again, are looking at diploid, or normal chromosomes, versus tetraploid. And we can see that the cells look even more different at this late time point. Over here are some pathways that are different between the diploid and tetraploid cells, and the red asterisks are denoting pathways that are commonly associated with Richter's transformation, that also are seen in our tetraploid cells.

And then, finally, when we look over time at just the tetraploid cells, we see evolution of the cells. So, genes become more upregulated over time, similar to what we have seen in the literature when we compare Richter's transformation cells to CLL cells.

So, what we're showing from this is that the tetraploid cells start out a little bit more – have gene associations that are typically seen with Richter's. We see those get even more pronounced over time. And so, one of the reasons to do this is to see whether there might be targets that could be used for therapy here. And one of the genes we see consistently overexpressed in our tetraploid cells is called PLK1. PLK1 is a gene that's very important in the regulation of cell division. It's actually dysregulated in a lot of cancers and is very tied to activity of TP53.

In addition to our primary CLL cells, our tetraploid cell lines also have higher expression of PLK1, which makes them a good mechanism to study this as a target. There's a number of PLK1 inhibitors in clinical development, although none have yet been tested in CLL.

Here's some data showing you that when we treat our cell lines, so our OSU-CLL cells that have normal chromosomes – that's the 2N, or tetraploid cells, 4N. And you can also see there are OSU-CLL cells that say p53 knockout. Those are actually also tetraploid cells that became tetraploid when we knocked out TP53. When we treat these OSU-CLL cells, or

MEK1 cells, with a PLP1 inhibitor, we see that all the cells really have some sensitivity to the drug. But those that are tetraploid, which are in red and in green, are increasingly sensitive to treatment.

And interestingly, when we treat our CLL cell lines with a PLK1 inhibitor, we can actually reverse some of the genetic changes that we see tetraploid cells acquiring over time, suggesting that we are making them act more like cells with normal chromosomes.

What we found so far is, tetraploidy is a major risk factor for Richter's transformation. That tetraploid cells develop gene abnormalities at a faster rate than those with normal chromosomes. In primary CLL cells, tetraploid cells are different than their diploid counterparts. They have gene expression that we associate with Richter's transformation, and they evolve over time. And PLK1 is a target that's overexpressed in tetraploid cells, and we think this might be an opportunity for intervention.

This is a project that is obviously still underway. And we're hoping that by understanding more and more about tetraploidy in both CLL and Richter's transformation, we can develop better therapies to treat Richter's, or potentially even prevent it from occurring. Thank you very much.

Dr. Catherine J. Wu: Well, it's a pleasure to be here, and to share with you some of our ongoing, exciting research. The title of my talk is "Targeting CLL with Cancer Vaccines," and this is a very exciting ongoing area of work. Recently, together with Dr. Wierda, Dr. Kipps, Michael Hallek, John Gribben, Nick Chiorazzi, and Federico Caligaris-Cappio, on behalf of the International Workshop in CLL, we put together a special report that is coming out soon in blood, about the research priorities in CLL. How can we get to a cure?

And what this image is trying to depict, which is part of the article, is that we've made a lot of progress at this point in 2026. And we've gotten to a place, shown on the left bank, where we've made so many strides in terms of how we think about the therapies that are available for CLL, how patients can be treated. But we really want to cross over to that next stage, which is, how can we precisely know what are the right therapies for which individuals with CLL, and how can we get there better and faster?

Together, we started to brainstorm, and we really think that the next frontiers are as labeled in the steps. Really understanding the, what we call the molecular taxonomy. So, how do we classify the different subtypes of disease? We have to understand what are the dependencies and vulnerabilities of CLL, and the basis of therapeutic resistance?

We have to also go back fundamentally, and understand, what are the origins? How do we get here? How did a normal B cell become a CLL cell, and even transform into Richter's? And finally, the fourth part is understanding that interaction between the CLL cells and the immune system.

And so, really, I want to highlight that this kind of immune interaction is something that we've known a lot about in CLL for some period of time. But with the better tools that we have, how can we precisely get to, what are those critical interactions? And in doing so, could we actually get to intervention at an early stage, so we can change the natural history of what happens when a patient has CLL? And so, that's just kind of a backdrop, and some understanding of where we are in the landscape of everything else going on.

This is a complicated image, but I want to note that we are in a very exciting time in general, in the therapy for cancer. Because immunology has really come to the fore. And there are, as noted here in the different quadrants, many different modalities of immune therapy that there might be.

There might be adoptive cellular therapy, there might be vaccines, there might be immune checkpoint blockade, or there might be T-cell antigen-directed antibodies. But at the center of all of this is some understanding of what is being displayed on the surface of CLL cells, that distinguish it as a malignant cell, not as a normal cell. And if we could understand what those so-called surface-expressed peptides, we call them antigens, are, that is the sharp point of the spear. That is how we launch our immune attack.

And so, in the vein of thinking about, how do we harness immune responses? How do we do it in an antigen-specific fashion? Well, cancer vaccines are really front and center of that effort. And cancer vaccines have been around the block for a long time, and they're very unique in their ability to modulate immune systems.

Because on the one hand, going from 9:00 to 12:00, they're really focused on the antigen, and trying to foster immunity in a very precise and potent fashion against those antigenic determinants. And if you target enough of them, maybe this can address whatever kind of variability is within the cancer population, so that we can avoid the subsequent arrival of resistance, which can happen when tumor heterogeneity is not addressed.

Going from noon to 3:00, we know that vaccines are a potent way to increase the quantity and quality of T cells. Going from 3 to 6:00, we also know that they can stimulate inflammation and the driving of interactions with the immune microenvironment. And we are always cognizant of how we can best deliver and manufacture these vaccines.

But I think a key takeaway for vaccines is, it is an opportunity to expand the T-cell reservoir and promote immune surveillance and memory. And so, they really have the ability to amplify pre-existing anti-tumor T-cell responses, but also prime and expand new anti-tumor responses.

And so, I alluded before to this roller coaster that we've been on. Vaccines have been interrogated over the decades, and sometimes, there's been a lot of excitement.

Sometimes, there's been a disinterest. But we are in a very exciting time, because the new technologies that have come upon the scene have really been transformative.

And I would say that in the past decade, we've observed a convergence of transformative technologies that have yielded new immune targets that we can go after. And this is what we call neoantigens. And this has really been a result of next-generation sequencing, so, NGS. This is because we have better neural network-based prediction algorithms that allow us to take that sequence information, and start to tile around mutations, and start to predict which ones out of the whole array are the ones that we want to go after.

And for more than a decade now, we've had FDA approvals of immune checkpoint blockade antibodies. And I like to talk about that as kind of like the Obi-Wan Kenobi moment, if you follow Star Wars. Think we all remember that moment when Obi-Wan Kenobi goes into the Death Star and goes, "Hmm." And then, the whole Raiders shield goes down, and suddenly, the Death Star becomes more vulnerable to attack. And that's really what I think immune checkpoint blockade antibodies have done for us.

And in terms of launching the attack, really, I think our fighter pilots are going after the neoantigens. And so, what are neoantigens? Well, these are targets that arise because of DNA mutations in tumors. And mutations are part and parcel of the identity of what a tumor is. We've learned so much from next-generation sequencing about finding and discovering what those mutations are, which proteins do they come from, and which ones are the ones that are really driving the generation of that type of cancer. Including for CLL, and in previous talks, I've talked about our work discovering DNA mutations in CLL.

But in this case, those DNA mutations also provide us with a new therapeutic opportunity. Because some of those mutations can lead to, from the DNA level, to a change in the RNA, a change in the protein level, so that we have altered peptides.

And at least some of those peptides have the ability, as they go through processing and presentation within the tumor cell, they can have the possibility of being bound to a patient's own HLA molecules. And HLA is the vehicle from which these peptides are seen by the immune system. So, when they hit the surface, at least some of those mutated peptides that are presented have the ability for, then, the T cells to come and recognize them.

And so, the concept of a neoantigen is not a new one. It's just that we never had the way to actually systematically find the mutations on a patient-by-patient basis. Now, problem solved with next-generation sequencing. And when this started to happen more than a decade ago, myself and other groups started to wonder, "Couldn't we just, in real time, start to analyze, at the DNA and RNA, level those tumor-specific mutations? And couldn't we use the HLA typing information, and those fancy epitope prediction tools, and start to ask for a patient's own set of mutations? What are the mutations that could generate

these so-called neoantigens?"

"And if we could identify the personal neoantigens for each individual, then could we not prepare a vaccine that could be directed and given, administered to patients, to mount T-cell responses against those neoantigens?" And this was the concept that we and others really started to test.

And so, what this graphic is showing is the immense progress that we've made over the last seven or so years in this area. Our first study, Ox Nature 2017, was the first of its kind that we co-published together with our colleague, Ugur Sahin, back in 2017. The way this graphic is organized, the left lane is for synthetic long peptides, SLPs. So, these were peptide vaccines. The middle lane is for RNA vaccines, and the right lane is for other types of vaccines. And what you can see is that, initially, we were focused on, can we even do it? Is this safe? Is this feasible? Answer is, for sure, yes.

And the next-level question was, in the blue font, "Were these actually able to stimulate a strong immune response?" And across many different tumor types, the answer, again, is for sure, yes. And since then, we and others have been able to show that we can demonstrate that such vaccines actually hit tumor and are able to target tumor.

What's been very exciting in the recent couple years is that the studies have become sophisticated enough, so that we can start to detect whether or not there's clinical impact. In the green font, what you can see is, in diseases as tough to treat as pancreatic cancer and high-risk renal cell carcinoma, that, in fact, when we give these vaccines, patients have a less chance of recurring after getting the vaccine. Been super exciting.

And that's the top of the road in the yellow, is that there's been a 150-person study, which is in a randomized open-label Phase II study in melanoma, that demonstrated benefit of adding a vaccine together with anti-PD1 therapy, compared to anti-PD1 therapy alone. And this is that information here.

So, on the red line is those patients who got both the mRNA vaccine that targeted 34 different targets, neoantigens, together with pembro. So, this was entirely a personalized vaccine. Each and every one of those tumors were analyzed in real time, and a personal vaccine devised for them. And those patients had less recurrence than those patients in blue, who received the antibody alone.

And this type of activity has really spurred a lot of excitement in the field. There are a lot of clinical trials right now, spearheaded by different companies. And what I want you to notice is that the original clinical trials that we and others did were in the academic settings, and they were studies of eight, 10, 15, 30 patients. But look now, we're getting to hundreds to thousands of patients that are being treated. And I do think that for these solid tumors, we're going to hear about the readouts, probably in another year or so.

For CLL, this is what we call a lower mutation burden tumor. And so, there, the number of neoantigens that we can target are understandably less, based on the DNA structure of the malignancy. So, what do we do, then? Is it so easy to find neoantigens that we can include in vaccines?

And certainly, there are challenges in T-cell immunotherapies for blood malignancies, and also, for chronic lymphocytic leukemia. We have to think about what are the right tumor antigens to go after? We're always dealing with cancer heterogeneity. And we think about the lack of knowledge of the properties of anti-tumor T cells associated with effective responses.

And then, we also think about the absence of effective, sustainable strategies to reprogram T cells for optimal persistence trafficking in the tumor, and ability to eliminate tumor cells. And so, I come back to that concept where it's really important to think about what the antigen is.

What this graphic is trying to show is that, in the current day and age, where we've really been is above the surface of the water in this iceberg. So, I talk to you about neoantigens, which are here on the left. Tumor-associated antigens is a class that we've thought about for decades. Not necessarily effective, but certainly out there. Some cancers are also generated by viruses, and there have been some promising work there.

But look at what's beneath the surface. There are so many parts of our genome that have not been heavily cataloged or characterized. We call it the "dark proteome," because when we look at the protein level, there is a lot of junk stuff that we haven't fully analyzed. And we and others have started to look at what is in the tumor immunopeptidome and trying to understand what the dark proteome is.

And I'm not going to go about this in detail, only to say that there are nascent efforts that are pretty interesting that are already going on in our groups, trying to understand this dark proteome, and to see what type of immune responses they can generate. And I do hope that in the future, in the not-too-distant future, actually, I think that what we have down here in the linear line on the bottom is what we are currently doing for our vaccine trials. Which is starting from tumor, and analyzing DNA and RNA, and then coming up with a set of neoantigens to put in our vaccines, to give to patients.

But what if we instead first did a mass spec characterization, which allows us to directly physically see, what are the peptides that are being produced by tumor cells? Which we can analyze directly with what we call this – by mass spectrometry. And then, this could help us inform what to put in our actual vials.

And so, this general excitement about where we are in the vaccine field has led us to

partner together closely with the National Institutes of Health. They tapped myself and other colleagues to put together a vaccine roadmap, so that we can really bring vaccines out to patients more broadly.

And I had the privilege back in December of assembling a fabulous team of experts in the field, represented across the country. And together, we started to identify what were the key questions that have been missing that we still need to address, in order to make cancer vaccines more broadly available. And on St. Patrick's Day, we were able to go to the National Cancer Institute, and this proposal was met with great enthusiasm. And there's an effort right now to raise 200 million in a public-private partnership, to fund clinical trials of vaccines.

And so, I do hope, and I'm quite optimistic, in the not-too-distant future, with all our bank of knowledge that we've learned from our research studies over the past one to two decades – especially since I do think that sequencing is, and should be part of, the routine diagnostic tests that patients get when they are first diagnosed with cancer. That we can then work with our primary oncologists and order up a cancer vaccine to be given. And that the pharmacies at our hospitals can say, "Sure. These will be ready in a few weeks."

So, aspirational for sure, but I don't think unrealistic. Thank you for your attention, and I hope we have the opportunity to be in contact in any time in the future, should you have questions.

Dr. Erin Parry: Thank you for the opportunity to be here today with you all, virtually. My name is Erin Parry, I'm at the Dana-Farber Cancer Institute, and I'm going to be talking about my CLL Global Research-funded project on defining evolution and dynamics of CLL transformation.

To start, what is transformation? Transformation is, on a basic level, a change in cancer cell type. Chronic lymphocytic leukemia, as you are all aware, is a typically indolent B-cell malignancy, although it can have a very variable disease course. And occasionally, for small subsets of patients, one of those CLL cells undergoes some dramatic changes that make it transform or become a different cancer cell type. And when this happens, this transformation is to a different kind of an aggressive lymphoma. We call this a Richter's transformation, or sometimes even a Richter's syndrome.

Why is this important for us to know, or understand, or study in the laboratory? Well, again, as many of you are aware, we have really made a lot of progress in CLL over the past decade or so. We have a number of really highly active targeted therapies, and others on the way, in clinical trials.

On the other hand, when we think of the transformation, this is a disease that typically has not responded as well to the therapies that we use for other types of regressive

lymphoma. And therefore, there's a lot of research that's needed to understand why it's different, and how we can better target it. And there's a lot of, also, exciting clinical trials going on in this space, so that we're hopefully starting to move the needle and make a lot of progress.

When I talk about this idea of transformation, I'm mostly talking about the type of transformation that's most common, which is when CLL goes to a large B-cell lymphoma. But I do want to acknowledge that there are more rare types of transformation that I won't be referring to today.

As we've made progress in understanding transformation, I like to show this slide because I think it highlights a lot of the work that's been done as a research community, just in the last five years.

So, we've started to be able to unlock, layer by layer, different insights into what is going on in terms of transformation. Understanding things like the genetics or epigenetics, which is sort of the packing and organization of the DNA, outside of mutations or changes in the DNA. Immune microenvironment stuff, and also, understanding how we can study these things outside the body using model organisms or different functional pathways.

But why has it taken us so long to get here? We are supposedly in the age of genomic medicine, where we're able to get sequencing in a rapid fashion. Many of you have even probably had next-generation sequencing from your physicians in the clinic. So, why do we know so little about transformation, given that these tools are readily at our disposal? And I think there are a couple unique features that made this a harder problem to crack until recently, and something that we're still learning a lot about.

One of these has to do with the idea of sample acquisition. So, to really properly understand transformation, we really need serial samples to see how these cancer cells change over time. We can't just have a single state; we need both states present. So, we really need samples from the CLL cells as well as the transformed cells, to understand what's dynamically changing between the two. This relies on us having robust biobanks, and individuals with CLL and Richter's transformation who are willing to share samples, and have these samples stored appropriately, so that they can be accessed by researchers.

In addition, when we think of biopsies that we get, they don't tend to look as much like these simple cartoons that are on the slide right now. They tend to look more like this, which is that an average biopsy, which usually is a lymph node or bone marrow tissue, is usually a jumbled-up mixture of different cell types. There can be normal cells, normal healthy immune cells, admixed with CLL cells and Richter cells, all in the same biopsy.

So, if we're looking at a single time point of a transformation, there are usually many cell

types present. And we're actually dealing with this issue of two different cancers, which complicate a lot of the traditional pipelines that are used for these types of analyses.

To highlight the first, I think, key finding, is collaboration and sample banking are really key to being able to study this problem. And we've taken the approach of using some of these annotated biobank samples, looking at normal samples, CLL samples, and Richter samples, in parallel with sequencing. And being able to apply this sort of detailed, long list of different computational tools, to basically get to the end result of being able to separate the CLL DNA changes and the Richter's DNA changes out.

You'll see that I refer to these, on this cartoon, as clones, and that is because cancer cells are really clones of each other. They look identical. And within a given cancer, we have these different subpopulations of different identical clones, as cancer itself is always constantly growing and changing.

So, what does this look like, and how can we then integrate this information? We do something that might be familiar to those of you that might do genealogy or know things about family ancestry. And that's, we try to make a phylogenetic tree. So, we're trying to put together these different cancer cell clones, and order them, and understand how they're related to each other. We're basically trying to reconstruct a family tree for an individual transformation event.

And so, you can see an example of this here, starting from something that is normal, to the earliest CLL cells that we find. And then, we can understand that some CLL cells, now progressing to the lighter green, ultimately are the ones that give rise to transformation, which is highlighted in the shades of pink and purple. While other CLL cells, or branches of the tree, sort of diverge, and are more like cousins to the transformation. They're not directly related to the transformation but are evolving in a different direction.

And so, we apply these kinds of approaches to every set of samples across an initial cohort of 50, and then in another validation set of 45, as well as an external cohort of 14, so that we were able to look at more than 100 different samples in our initial study. This allowed us to get some key insights into some of the different molecular or genetic alterations that were happening. These are the type of genetic alterations that are different, that set cancer cells aside from those of normal cells.

And we could find that some of these impacted some of the common pathways in CLL. Some, for example, like NOTCH mutations, were early CLL events. Others, such as those impacting DNA damage and some pathways of MEK activation, tended to happen in those slightly later-evolving CLL clones that were getting closer towards transformation.

And then, around transformation, there were a lot of new events occurring. Many of these involved in pathways that allowed the cells to escape from the immune system.

Pathways that allowed the cells to suddenly start dividing more than they should and impacting those breaks that are on the cell cycle, and on cell division, typically, as well as things involving inflammation, and as I mentioned, epigenetics, are the things that aren't encoded in the DNA, but are important in terms of regulating the DNA, and how it's organized.

And this correlated, too, with what we call different regulatory pathways. So, moving from the level of DNA, to thinking of how those DNA genes are actually expressed. And this led us, again, to these similar pathways of inflammation and cell cycle, as well as some pathways of altered cell metabolism.

And this data was not only seen in our study, but also a couple other studies that were done around the same time. Lending a lot of robustness and providing us with a really unified understanding of some of the changes that are going on during this process. But there are still a number of open clinical questions. And as we strive to make things better with individuals with CLL and Richter's constantly, here are a number of the areas that we're trying to address with our current research.

First, what's the diagnosis? If you know of anyone who's undergone a biopsy for ruling out transformation, or ruling in transformation, it can be a complicated process. Not only do we rely on specialized scans to try to target the biopsy, and it's not always straightforward to be able to get the tissue that we need. But there's also a process of the pathologists, which are the doctors that look under the microscope, being able to look at the tissue and tell us that, what we are actually seeing.

And so, there's a spectrum of the way that CLL even appears. And being able to diagnose it with infiltration of large cells, which is what we need for transformation, is not always straightforward. So, if we could get to a place to aid in that diagnosis, make it simple, more streamlined, and perhaps even non-invasive, I think this would be really impactful in the clinic.

In addition, thinking of who is going to get transformation. It's only a small subset of our patients with CLL that ever experience a transformation event. And if we could predict who is most likely to have that happen, we could reassure the vast majority of patients that that is really unlikely for them, based on their individualized risk profile, and perhaps follow those patients differently that are at the most high risk.

And leading along those same lines, when does transformation happen? Is there a certain point at which a CLL cell gets committed along that pathway? And if that's something that we could detect and intercept, or know when it's going to happen, then we could perhaps intervene with therapies before people get sick. And perhaps divert these clones away from that pathway, so that we could actually prevent it from happening in the first place.

In order to start seeing how we could get to those places, the focus of my CLL Global Research, currently, is looking at many of these single cells and trying to understand patterns of genetic evolution better. We started by looking at samples as a whole, and now that our technologies are getting better, we're looking with deeper and deeper lenses to be able to see things that we were missing in our initial studies. Because we couldn't look with a big enough magnifying glass, so to speak.

So, we're looking, now, at single-cell DNA and RNA sequencing approaches. So, we're able to see the genetic alterations, or the gene expression changes, or the DNA packaging changes, within a single cell. And we can use this information to help us similarly reconstruct those family trees to understand cancer cell clonal evolution, and understand how, in some patients, that CLL cells could transform into a Richter's transformation.

And so, we're taking this approach of looking at serial samples. And so, you can see in this cartoon format, you see a cartoon of serial samples where you see different color cells. So, the changes of different cancer cell clones over time. And we're capturing these different unique populations in serial samples, and then asking, "What are the unique populations? What are their properties, and how are they related?" In other words, can we make that family tree?

And so, in our initial attempts at this, this is sort of early data, we found a couple of really unique things that allowed us to proceed further. One is that because these cell changes are so different, because there's a fundamental switch in cancer cell type, they're very different. And so, when we conduct sequencing using RNA, which is the gene expression, the cells really separate.

So, you can see these colored blobs. But if you can look back, you almost see two different-colored blobs. One of those large blobs made up of different colors is CLL, and the other multicolored blob is actually Richter cells. So, they separate from each other, which allows us to distinguish them readily by their gene expression profile. We also saw that there were some populations that were kind of on the fence. These looked like a little bit of an in-between genetic step but also had in-between transcriptome profiles. This is pictured by Cluster 7 and Clusters 4 here, if you can see the numbers.

And then, we could also find some populations that were really small. So, things that would be below the resolution of what we can find in a bulk sequencing sample, where we're sort of limited, and it's hard to pick things up that are really rare. And we could find these really rare populations when we're looking at single cells. But there were initial limitations. And these first attempts at this, that were published a couple years ago, were limited by resolution.

We also had an issue with what we call data sparsity, meaning, our sensitivity in detecting things was low. So, if they were there, and we found them, that was great. But sometimes,

they were there, and we just weren't able to detect them. We had dropouts or missing values. And also, initial assays were single data levels. And remember that initial figure where I showed you integrating different data layers? Ideally, we'd be able to get multiple kinds of information from one of these single cells.

So, how did we improve upon this? We first looked with a combination of looking both at that gene expression RNA assay, but as well as looking at some of the DNA accessibility or packaging. And we integrated these data with this genetic tool called Numbat-multiome, that was developed by some of our computational biologist collaborators. We also did genome sequencing in parallel to make sure that we were on the right track.

And what we did, and how we were able to integrate these, was to take advantage of little variations that we all have in our DNA. These are things that we inherit from our parents and aren't related to cancer. They're normal benign variants that we all have, that are some of the things that make us different. And by looking at these different things, it allowed us to be able to detect how copy number aberration went wrong in the cancer cells compared to an individual's normal cells. And so, allowed us to detect things and to reconstruct these trees. I'm going to show you an example of this now.

In each one of these different-colored circles is one of these cancer clones, or a stop on the tree. And you can see that the black lines that are now connecting these is an example of one of those trees. Where you can see that there's an original clone that gives rise to a side branch that's green, and then a side branch that's blue, and that blue side branch gives rise to the transformation clone in pink.

And now, when you look at these really complicated plots with lots of dots that are next to them, these are actually showing, from one chromosome all the way to the end of your chromosomes, the copy number profiles. And so, starting at the top, there's very little copy number. And as we move towards the bottom, we see more and more copy number, which is shown in green, in red, and in blue. And these copy number alterations, including complex ones, are ones that help define or mark these clones, and allow us to trace them. And then, we can, in turn, look at what is unique about these clones, and when they're occurring.

And what we found is, all of these clones were pre-existing, for instance, before therapies start. But the Richter's clone really emerged and newly developed from one of those pre-existing clones after therapy started. And you can see a little bit of how this looks on this map. This is another cartoon version of this trajectory, or evolution, or tree, occurring in real time.

And then, also, we are able to infer or determine which transcription factors. These are like master switches in the cell that help turn things on or turn things off. And in this particular case, we could see how sequential changes in these was really helping change

that global signaling and behavior of the cell and helping it really reprogram into a different cell type.

This started giving us a lot more information. So, we're on the right track. But how does DNA sequencing compare? I'm telling you about all these other data layers. But what if we go back to the DNA layer? And the bottom line is, we actually have more evolutionary stops on the tree. So, using DNA, and being able to do sequencing of the DNA level as opposed to those other assays, gives us a tree that has eight clones as opposed to four. So, even more steps in evolution, even more in between to see. But this is only DNA.

So, going back to that same question, how do we combine more data layers? How do we get DNA, and RNA, and things like that, in a single cell? And so, to do this, we've partnered with collaborators of ours at the Broad Institute, where they've developed new technology to be able to do just that. And so, we've designed customized panels and approaches that are sort of unique to being able to study CLL and transformation. And one example of this is on this slide, but we're now able to get RNA and DNA in single cells.

So, this is just a single time point, for instance, where we're able to pick up already normal cells in four different distinct clones of CLL. And now, we can link their clonal phenotype and clonal properties, so we can see each step or each change in DNA, how that reprograms the cell and allows it to behave differently. So, now, for the first time, we're being able to really link cancer cell genetics to how they behave, and this is going to hopefully give us a great, deeper understanding into the process of transformation.

In conclusion, we're working on these new tools and technologies, and being able to leverage them to better understand this process of longitudinal CLL evolution, including to transformation. And as part of our long-term goals, we really want to be able to work towards an improved diagnosis and recognition of transformation, to be able to intercept, and identify, and then even prevent transformation.

And ultimately, as a community, all of us researchers are really working hard to make sure that we can continually dedicate our efforts to improving outcomes and quality of life for individuals with CLL and transformation.

Thank you for your attention. I have lots of people to thank, but first and foremost, want to thank the CLL Global Research Foundation for their support, and for being part of this really wonderful community of scientists, and researchers, and physicians, all dedicated to improving things for CLL.

Prof Constantine Tam: Hi, my name is Con Tam. I am a hematologist in Melbourne, Australia, and a CLL researcher. And it gives me really great pleasure to speak to you today about some of the progress, especially at the more difficult end of CLL for patients with resistant disease, and for patients with a rare complication of CLL called Richter's

transformation.

Within Australia, in Melbourne, we've been very lucky recipients of the funding from CLL Global, which was started by Professor Michael Keating, my mentor, and continued by Professor William, Bill Wierda. CLL Global Research Foundation has funded us in Australia, my group in particular, for research, for six years now. And shown here are the papers that we have published, based on the funding that were very generously granted to us by you, the patient, to allow us to fund the CLL research.

As you can see, there are a number of papers here. I struggled to fit the titles on the screen. But essentially, we have come a very long way in understanding how our drugs work in CLL, how to better use our drugs and new technology to monitor CLL. And I want to say that much of the huge leaps and progress that we've made in the last 10 years in CLL were thanks to the funding from the research foundation. So, a real tribute to all of you for helping to fund this work.

I want to start by telling you a story about some of the work that we've done so far, especially in patients with resistant CLL. You will be aware, as a patient, that there have been some real successes in targeted therapy for CLL. One class of drug on the left are the BTK inhibitors, of which the first member was ibrutinib. And some of you may have experienced ibrutinib before. But now, we're moved on to zanubrutinib (Brukinsa), acalabrutinib (Calquence), pirtobrutinib (Jaypirca), and newer generations. But really, these have been a real pillar in CLL therapy.

And on the right, there is a second group of drugs, a drug called venetoclax (Venclexta), which inhibits a second protein called BCL2. And once again, this had been a very important pillar in CLL therapy. And these days, as a patient with CLL, patients would typically get both classes of drugs at some time in their lifetime. Either one after the other sequentially, or in some cases, receiving both at the same time.

But really, these are the two main pillars of CLL therapy these days, and probably the largest group of patients. The very few patients who have disease that became resistant to both this type of treatment, other type of patients where we are having the most trouble with these days, and that's where we're focusing our research to develop new therapies. But these two groups of drugs have completely changed the way CLL has been managed. We do not give chemotherapy anymore because both these groups of drugs perform better than chemotherapy.

Our group's very interested in finding what causes venetoclax resistance. And we're very lucky, in 2018, to have discovered the mechanism for venetoclax resistance. And this is a change in the protein where venetoclax binds, and a change in the spelling sequence of the protein, which stops the drug from binding. These are some of the results that we have shown.

But we've actually done a lot more work on understanding why patients become resistant to venetoclax. Because if we take a typical patient who has this mutation that stops venetoclax from binding, that mutation is only in a small percentage of cells in the CLL. And the rest of the CLL cells actually do not carry that mutation, and should, in principle, still be sensitive to the drug.

Our group has done some really interesting work on something called single-cell sequencing, which is when we look at the individual cells within a CLL patient to understand, not whether the patient has a mutation or not. But at the individual cell level, we look at each individual cancer cell, whether they actually carry the resistant mutation or not.

And very interestingly, we're discovering that not only, some of the cells do carry the resistant mutation, but other cells within that same patient will carry other mechanisms of resistance which will still describe it. And, of course, this is a really important piece of work. Because if we now understand that a patient has developed resistance to a drug because of a mutation, but the mutation is only in a minority of the cells, and we target that mutation, then we're ignoring why the rest of the cells are becoming resistant.

So, we're now working on understanding the entire landscape of venetoclax resistance, so that we can develop new treatment that will cover both the cells that have the resistant mutations and the cells that do not. This is a work in progress, but we've made huge leaps in progress so far.

Exciting developments spurred on by the venetoclax story is a drug called BGB-11417, or sonrotoclax (Beqalzi). This is a drug that is 10 times more potent than venetoclax, and recently has received FDA approval, starting from a, first, in human phase at our group. And this is a drug that may be coming to you soon, as a patient.

And what this drug does is, it is so potent that it's able to overcome some of the venetoclax-associated mutations. Although, as I pointed out before, that there are other mechanisms of resistance that we still need to address. So, this is one step towards understanding and overcoming venetoclax resistance.

On the other hand, on the ibrutinib, on the BTK side, we have made very large leaps in progress. And we now understand, in most patients, what causes a resistance to a BTK inhibitor. And we now have at least two new generations of drugs to overcome that resistance.

This is not our work; this is work from the U.S. group that showed initially that for patients on ibrutinib long term, the first-generation drug, then most patients who become resistant have a mutation at exactly the place where ibrutinib binds, and this is in a place

called cysteine 481.

Now, this is a really important mutation, because for subsequent drugs like acalabrutinib and zanubrutinib, they all bind to the same place. So, if you have this mutation, you are resistant not only to ibrutinib, but also to zanubrutinib and acalabrutinib.

Our specific group have contributed to development of the second-generation drugs. So, we worked very hard, and, in fact, I was very lucky to be the principal investigator of zanubrutinib. And as you know, zanubrutinib is a drug that is more potent than ibrutinib. It worked better, and it has less side effect. And similarly, for acalabrutinib, these are all second-generation drugs, which have less side effect. But they all bind to the same place.

Now, one discovery that we made some years ago, founded by the CLL Global Research Foundation, was that patients on zanubrutinib actually carry a second mutation in a place called L5 A28W. And for acalabrutinib, there's a second mutation called the T474I. Now, these mutations were not just of academic interest. We love CLL, and we're geeks, but these mutations are really important. Because what they do is they actually confirm resistance to pirtobrutinib.

So, now, our eyes have been opened. When we're talking about BTK mutation, we do not need to consider just the 1681 mutation, but, in fact, a whole list of other mutations, in order to best choose the drug that's likely to work for our patients. Especially if you are going to use one drug after another.

But the latest chapter in that story is that we have now been working with a BeOne company to work on this drug called BGB-16673, which is actually a fourth-generation BTK inhibitor. And what this drug does is, it doesn't just block BTK; it destroys the protein. So, all the previous generations of drugs block BTK function, but this is a protein degrader. It destroys the protein. And the advantage of that mechanism is that if you destroy the protein, then it overcomes most of the resistant problems with the resistant mutations.

And indeed, I want to highlight here, in a early report, that, down at the bottom, that a patient that we treated on this study, even the people who carry a resistant mutation in BTK, there's a three and four chance that that patient will have a really good response to BGB-16673, which is a next-generation degraded drug.

So, once again, just a flavor that we are understanding what causes resistance to drugs, and we're finding ways to overcome them by understanding, in detail, the science behind the mutations. This is to show that patients who carry mutation have just as long as a response, and as durable as response, as patients without.

Now, I want to move quickly onto Richter's transformation, because this is the new frontier where we really need to devote our funding and our research efforts towards.

What is Richter transformation? In about five to 10 percent of patients with CLL, the disease no longer uses CLL. After a while, it becomes an aggressive, fast-growing lymphoma, something called diffuse large B-cell lymphoma. And when that happens, it's very bad. Because usually, Richter's transformation is resistant to all the drugs that we use, including BTK and BCL2 inhibitors.

This is just to show that if you have – we typically try and treat these patients with Richter's transformation with old-fashioned chemotherapy, and the various regimens are listed there. But what I want to point out on the right is the average survival of the people who are treated with chemotherapy for Richter's transformation in different studies. And you can see that, really, the outcome is pretty terrible.

For someone, if you've got Richter's transformation, and you get typical chemotherapy and nothing else, you're looking at an average lifespan of about six to 12 months, which is why it is such an urgent problem.

A progress in Richter's transformation is that we have now developed what we call bispecific antibodies. And, in fact, this is a group of drugs that was identified by Dr. Keating as being promising in CLL over 20 years ago. And what these drugs do is, they bring the immune system to the cancer.

The one that we're looking at here is acalabrutinib, and what this drug does is, it handcuffs the patient's immune system to the cancer, and it forces the immune system to attack the cancer. And in early studies of patients with Richter's transformation, which is a very aggressive disease, we see that there's about a 50 percent chance of the patient going into a complete remission, meaning that the cancer is completely destroyed by this therapy.

In Australia, what we've done, the other thing that we've done in Australia is that we have now introduced CAR-T cells for Richter's transformation. And a lot of you may have heard of CAR-T cells because this is something that we use for aggressive lymphomas. And in Australia, we're very lucky to have actually got access to CAR-T cells for Richter transformation. And this is survival curve of the latest generation of CAR-T cells in the red line, which is with axis cell.

And what this survival curve tells us is that probably around half the patients with Richter's transformation, even when they don't respond to chemotherapy, can be saved by CAR-T cells, and potentially have a chance to cure. So, this is, once again, a proof of principle that Richter's transformation do not respond to the typical CLL drugs, do not respond well to chemotherapy, but can be effectively destroyed by drugs which target the immune system. Including the bispecific antibodies, as well as CAR-T cells, which harnesses the power of the immune system.

And obviously, this is only the first chapter in a big bulk of our work that we're still developing. But showing you, there is a glimmer of hope. And we now understand, have good ideas about how we need to target this difficult disease.

I hope I've given you flavor to show that, although most patients who have CLL these days do really well with first and second-line treatment options that we have developed with the help of the foundation, we understand that, to move beyond that, we need to understand the mechanisms of resistance to our drugs, which we have made a lot of progress towards, and we have developed third and fourth-generation drug, and that Richter's transformation remains a bugbear. But we're getting close. We're getting close to solving this problem.

And I want to thank you for your interest, and I want to point out that we're only here because of the support for research. And I want to thank the CLL Global Research Foundation for their very generous funding of our work here in Australia. We hope to work together hand in hand to finally cure this disease for all of you. Thank you.