

Fresh incentive to look for Ph-like ALL

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October 2018—Cheryl Willman, MD, could hardly believe her eyes. She and her colleagues at the University of New Mexico, working with collaborators from across the U.S. in the NCI TARGET Project, had submitted 100 cases of high-risk pediatric acute lymphoblastic leukemia to British Columbia's Cancer Agency for RNA sequencing to figure out why patients were doing so poorly, despite treatment with intensive chemotherapy. Now the results were in.

Dr. Willman, the Maurice and Marguerite Liberman distinguished chair in cancer research and UNM distinguished professor of pathology, vividly recalls the scene. Her own lab had discovered that a number of these cases of ALL had a gene expression signature that reflected activation of tyrosine kinase signaling pathways, and subsequent DNA sequencing turned up the *JAK* mutation.

But the results from Canada turned their heads: There appeared to be a large number of sequences that result from the fusion of various tyrosine kinases with other genes, many of them novel—which didn't seem possible.



Ph-like acute lymphoblastic leukemia isn't new, but interest is growing and much uncertainty remains about testing. "People are beginning to think of this disease now and want to do a screen to make sure they get that child on a trial," says Dr. Cheryl Willman, shown here at UNM with Dr. Richard Harvey. [Photo by Steven St. John]

"We actually questioned whether these were sequencing errors," says Dr. Willman, who is also director and CEO, UNM Comprehensive Cancer Center. "When they sent that first data set back, we actually thought it was a complete artifact." She laughs. "I'm not kidding."

A UNM colleague recalls his own amazement. "I was surprised there were so many cryptic translocations that existed," says Richard Harvey, PhD. "They all expressed with the same signature and had such adverse outcomes," says Dr. Harvey, research professor in pathology and member, Comprehensive Cancer Center.

Convinced that the sheer number of gene fusions was highly unlikely if not impossible—"We just couldn't believe

it," Dr. Willman says—the researchers passed a month arguing about whether the findings were indeed accurate, or whether they were simply artifact of either the polymerase or the (then relatively new) sequencing technology. They then spent a couple of months performing DNA sequencing to validate that the fusions were real. "Which they were."

And so began—and so continues—a new era in ALL, one in which a dizzying array of lesions could help open the door to more effective treatments, including use of targeted kinase inhibitors.

Leading the charge is Philadelphia chromosome-like ALL (also known as *BCR-ABL*-like ALL), which the WHO classified as a provisional category for ALL in 2016. It's only one of the multiple new subtypes that are being teased out among the 30 to 50 percent (depending on age) of patients with ALL who previously had not had a known molecular subtype. But because Ph-like ALL is associated with poor outcomes and can potentially be targeted with TKIs, "It's on people's radars," says Charles Mullighan, MBBS, MD, member, Department of Pathology, and co-leader of the hematological malignancies program, St. Jude Children's Research Hospital. He is a lead collaborator in the NCI TARGET Project (Therapeutically Applicable Research to Generate Effective Treatments).

And though the disease isn't new, much uncertainty remains about testing. How do labs identify whether someone has Ph-like ALL? And how should they identify the underlying genetic changes that could be targetable?

"We still get lots of questions from pathologists, because this is an incredibly complicated topic," says Dr. Willman.

A common struggle for nearly everyone, says Dr. Mullighan, is that the disease is not characterized by a single, named genetic lesion. Rather, the gene expression profiles of these cases are very similar to cases that have *BCR-ABL1*, which is the product of the Philadelphia chromosome. "How you define the gene expression profile can downstream influence whether you call someone Ph-like," Dr. Mullighan notes.



Dr. Hunger

A number of genomic studies have shown that most of these cases have acquired genomic alterations that activate kinase and cytokine receptor signaling, says Stephen Hunger, MD, Jeffrey E. Perelman distinguished chair in pediatrics; chief, Division of Pediatric Oncology; and director, Center for Childhood Cancer Research, Children's Hospital of Philadelphia. These fall into several *ABL*-class functions that resemble *BCR-ABL1*, and involve fusion of genes to one of several kinase genes, including *ABL1* itself, *ABL2*, *PDGFRB*, and *CSF1R*. In vitro, these fusions basically phenocopy *BCR-ABL1* and respond well to *ABL*-class tyrosine kinase inhibitors, such as imatinib and dasatinib. "There are anecdotal reports of dramatic responses" when TKI inhibitor therapy is added to these patients' regimens.

Another large class leads to alterations that affect *JAK-STAT* signaling, including rearrangements of the *CRLF2* cytokine receptor, and also alterations that cause fusions of *JAK2* or truncating rearrangements of the erythropoietin receptor. "Many of these are also transforming in vitro and will respond in vitro to *JAK2* inhibition with drugs such as ruxolitinib," says Dr. Hunger, though he cautions that "for this class of lesions, the responses in vitro seem to be much more variable than they do for the *ABL*-class lesions."

The underlying lesion is key. "Because there are literally hundreds of different genomic rearrangements that lead to these different phenotypes, then it's not as simple to diagnose as Philadelphia chromosome-positive ALL is," says Dr. Hunger. "And there have been a number of efforts to develop pathways to screen for the underlying lesions, because the Philadelphia chromosome-like gene expression profile itself is not targetable. It doesn't tell

you how to treat the patient.”

There are several options for identifying Ph-like ALL and underlying lesions. What you use “very much depends on your goal,” says Dr. Mullighan. In some cases, clinicians may want to identify cases that might benefit from dasatinib. In other cases, they may want to identify only lesions that are druggable with ruxolitinib. “Or do I want to find everything?” he asks. The first two groups make up about 60 percent of Ph-like ALL, he notes. Other lesions and pathways can certainly be identified, but they can’t necessarily be targeted by an approved TKI.

A word on genetics might be useful, Dr. Mullighan continues. “Unfortunately, the genetics of Ph-like ALL are very complex and diverse,” he says. There are well over 60 different rearrangements targeting 16 different genes. Some of them are one kinase rearranged to a great diversity of fusion partners. There are also a number of sequence mutations and copy number changes in DNA—particularly deletions—that also play a role in driving signaling.

At St. Jude, Dr. Mullighan says, testing is done at the most comprehensive end of the spectrum, with transcriptome sequencing and whole exomic genome sequencing. This approach is challenging, but “We’re not left in the position of having to circle back [for] retesting and other approaches when, for example, a more simple focus doesn’t give us a result.”

Even without such a comprehensive (and admittedly not widely available) approach, says Dr. Mullighan, many key lesions can be detected relatively easily. A rearrangement involving the *CRLF2* gene can be detected easily by standard tests such as FISH; similarly, flow cytometry can be used to detect overexpression of receptors on the cell surface.

None of this testing will let you know if the patient has the gene expression profile, however. That can be done by RNA sequencing.

Dr. Mullighan speaks on this topic frequently, covering the above basics. He jokes that after presenting at conferences, he often finds himself thinking, as he steps down from the podium, *I didn’t explain that very well*. “Because sometimes people still say to me afterwards, ‘But what test do I need?’”

It’s still hard, he says, for physicians to grasp that Ph-like ALL is not a one-size-fits-all diagnosis. “It’s actually a collection of different leukemias that have some similarity,” not unlike a family that shares, say, a penchant for German-style potato salad (boo, mayonnaise!) but can’t be trusted to talk politics around the picnic table.

Dr. Mullighan also urges his colleagues to think about when they test. “Don’t wait for failure. Clinicians should be thinking about this when every new patient comes through the door, thinking about options for targeted therapy early in treatment, and not waiting for relapse.”

Clearly, this is a fertile time for ALL care and research, Dr. Mullighan says. “And we continue to revise the taxonomy of the disease—that’s changed hugely over the last few years” with the identification of multiple subtypes and fleshing out how those subtypes look across the age spectrum. “There’s still work to be done,” he says. “But we’re getting there.”

Indeed, researchers have identified more than 20 subtypes. “That sounds daunting, and I guess in some ways it is,” Dr. Mullighan says. “But it’s also satisfying knowing that we can identify groups for most patients” and indicate, with some clarity, risks and possible treatment options associated with each. “There is a lot of excitement in the field, especially with adults, because it’s resolving what was unknown about adult ALL.”

Dr. Harvey seconds that. The head-turning work at UNM helped show, among other things, “that adult ALL was so much more similar to childhood ALL than had ever been appreciated.” The assumption that the two groups occupied different worlds “was my belief up until we first saw this,” he says.

That work also shed light on possible ethnic differences. At the time, Dr. Willman was running a reference laboratory and biobank for the NCI’s Children’s Oncology Group. While 80 to 90 percent of children with ALL

respond well to treatment, “It was clear to me, coming from New Mexico, where we have a large predominance of Hispanic and American Indian children, that those children were not doing well according to current dose-intensive therapies.”

They began by taking samples from children who failed treatment and looking at the molecular characterization of about 1,000 cases, starting with gene expression profiling and ultimately moving to transcriptomic RNA seq or whole exome sequencing.

“The interesting thing about the children who failed therapy was that their genetic lesions weren’t known in the majority of cases,” she says. “So we thought it would be a particularly fruitful group to study.” The project was picked up by the NCI and evolved into the first TARGET program—the first pediatric cancer project under The Cancer Genome Atlas—with researchers from UNM, St. Jude, COG, and Children’s Hospital of Philadelphia.

Some 15 to 20 percent of the high-risk kids, it turned out, had a gene expression signature barely reminiscent of *BCR-ABL1*. As their work progressed, they also found, through directed gene sequencing, that several of the children had a mutation in the JAK2 tyrosine kinase that is often mutated in myelodysplastic syndrome—but this one appeared in a different region of the gene.

But the real bombshell was the transcriptomic sequencing results. “We were completely blown away. It was almost like every child was different,” says Dr. Willman, with their own, specific tyrosine kinase fusion or point mutation. The number of variants currently sits at about 200. “It’s dramatic,” she says. Thirteen different kinase mutations have been described, with 48 (emphasis hers) different fusion gene partners. “It’s just sort of shocking.” And none of them recur frequently. “In other words, it’s not like 50 percent of the kids have one of these. They’re spread out,” not unlike people in Chicago who vote Republican.

Subsequent sequencing work had led to a gene expression profile that has been developed into a clinical diagnostic assay used for screening. “We’ve done a lot of testing now of more than 8,000 ALL cases of this type, from children, adolescents, and adults,” she says.

The test is a gene expression signature for either an eight- or 15-gene signature (which incorporates the eight genes). It’s FDA approved in the context of clinical trials. UNM is in the process of licensing and commercializing the screen.

Interest is growing, Dr. Willman reports. “We continue to get calls from pathologists and clinicians as they become more aware of Ph-like ALL.” This often comes up when a patient is not attaining remission on standard induction therapy, “which is pretty rare these days in the treatment of leukemia,” she says. They may also have a high white count, or (as noted) ethnicity might offer a clue. (She notes that the *GATA3* risk allele variant frequency is three- to fourfold higher in Hispanics and Indigenous Americans than whites.) Another clue: absence of the classic recurrent cytogenetic abnormalities pathologists typically see in other leukemia subtypes, such as a t(12;21) or a t(9;22). “People are beginning to think of this disease now and want to do a screen to make sure they get that child on a trial. We spend a lot of time on the phone with pathology groups,” Dr. Willman says.

Dr. Hunger fields plenty of calls himself. Ideally, he says, patients will undergo real-time screening using the new assay, “which we think is pretty accurate. And we have the ability then to enroll patients in clinical trials.” Here he interjects a note of caution. “At least from my perspective, the targeted therapies have not yet been proven to be effective. There’s a reasonable hope they will be, but clinical trials are needed to prove that.”

“I think the more common challenge,” he continues, “is the patient’s not enrolled in a clinical trial, and one of these abnormalities is identified by one of a variety of methodologies.” Next steps depend on how a patient is responding to standard therapy. “If they’ve responded very well, often physicians will keep this knowledge in their back pocket to use potentially if the patient were to relapse.”

The newer subtypes of ALL have an increased risk of relapse, he says. “A higher percentage of them will relapse than other, if you will, garden-variety ALL cases.” But not all will relapse, either. In particular, children with low-risk

clinical features do quite well with conventional chemotherapy, even if they have a high-risk genetic abnormality.

Dr. Willman seconds the importance of accruing patients to one of the national clinical trials. Not only are molecular tests being developed in the context of these trials, but given the likelihood of eventual relapse, “they’re going to need another round of testing to determine what to do next.”

Treatments are initially dramatic, says Dr. Willman—typically two to three years remission, “and then you begin to get clonal evolution and relapse.” Clearly the targeted therapies are essential, but so too is monitoring.

One of the most powerful tools available in treating ALL is the measurement of minimal residual disease, or, as it’s increasingly known, measurable residual disease, says Dr. Mullighan. Typically results are mutation agnostic, he says, using leukemia immunophenotype and flow cytometry, or detecting antigen receptor rearrangements quantitatively, to track leukemia burden. “That information is one of the strongest predictors of outcome,” he says, “and it’s used to risk-adapt therapy—if patients respond poorly, for example, [it’s] intensified.

“Where things are changing now,” Dr. Mullighan continues, “is how to use the new genomic information to influence the MRD testing.” Some fusions, for example, are being used as MRD assays (though not in Ph-like ALL). “You can already think that there might be a way you could track the specific rearrangement again—that would be flow cytometry to *CRLF2* because if that’s part of the leukemia phenotype, you conveniently track that clone by flow cytometry over time.”

Given the diversity of mutations, no single, standard assay will likely emerge for testing MRD, says Dr. Willman. “In a lot of our clinical trials, [MRD] is being monitored using flow cytometric testing, which makes sense—when a new clone emerges, you can pick it up” and then submit it for another round of detailed molecular testing.

The other complexity, Dr. Mullighan says, is that kinase lesions can be subclonal events and can wax and wane over time. “We often make a decision to intervene, with a TKI or a more intensive approach, at completion of induction therapy. The question then arises, if you’re detecting positive MRD after a month of therapy, are the genetics in that sample going to be the same as what we found in the original diagnosis? Or will they have started to evolve? Could that impact the decision to use a TKI?” Dr. Mullighan and his colleagues are addressing this prospectively in a clinical trial. “That’s by no means standard of care anywhere, to my knowledge,” he says. “But it’s a very important point—how do you build mutation-specific MRD tests?”

Meanwhile, the hunt for new fusions continues apace. For known kinases or cytokine receptors that are rearranged, “We continue to find more fusion paths,” says Dr. Mullighan, including, recently, an *FLT3* fusion that hadn’t been previously described in any cancer.

He and his colleagues have also taken a subset of cases, performing gene expression profiling to determine the group of cases that have Ph-like ALL, then applied other levels of sequencing. “We still can’t find the driver lesion. There are some cases that look, for all intents and purposes, extremely, strongly, Ph-like—yet we haven’t found a lesion. We are continuing to use whole genome sequencing and other approaches to try to find those drivers.”

“There have been a few surprises as this work has unfolded,” Dr. Hunger agrees. “One is just the diverse variety of genetic abnormalities that seem to get you to the same endpoint.”

“We continue to describe—this amazes me—new variants every month,” says Dr. Willman, sounding a bit like a highly caffeinated Adam, naming the inhabitants of the newly formed Earth far into the book of Genesis.

She finds this especially satisfying given her entree into the field. “When I submitted my first director’s challenge grant to do genomic characterization and sequencing in the acute leukemias, one of the reviewers said, ‘Oh, that’s stupid. Why would we fund leukemia? We know all those translocations already. This is a waste of money.’”

Though she didn’t share that misdirected certainty, she does concede surprise of her own. “I don’t think I expected that it would sort of expand or open up to such a broad discovery of so many genetic lesions.”

She’s also intrigued as she watches other researchers do transcriptomic sequencing in solid tumors. “They’re also

finding these fusion genes. We're discovering a much more complex spectrum of gene fusions present in these cancers than anyone ever thought. I think we were kind of one of those first windows into how complex the genomic lesions are in any one cancer. You don't just have one mutation or one amplification or one fusion gene. There are actually several."

The questions continue to emerge alongside each new discovery. "Is this the same clone that has all these mutations, or do we have multiple subclones that have slightly different constellations and mutations?" Dr. Willman asks. "I think the deeper sequencing studies have shown the latter to be the case. That most human cancers, even these leukemias I'm talking about, are highly clonally heterogeneous. As you begin to apply a therapy, and cause genetic evolution, you're actually doing that with the therapy, probably clonal selection and evolution—you're perturbing that heterogeneous mix."

The only unpromising note in this story, she suggests, might be the name of the disease that is transforming ALL diagnostics. Philadelphia chromosome-like acute lymphoblastic leukemia hardly rolls off the tongue. And unlike the arts, where a clunky working title can eventually give way to one that shines— isn't "The Sound of Music" more pleasing than "The Story of the Trapp Family Singers"?—medical nomenclature might be a little less flexible.

"We called it a terrible name," she says. "None of us like it, but it sort of stuck. Unfortunately."

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