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Tracking Lineage Infidelity in Pediatric B-ALL: New Insights From ASH

Ryan Quigley:
This is *Project Oncology* on ReachMD. I'm Ryan Quigley, and today, we're taking a closer look at new research presented at the 2025 American Society of Hematology Annual Meeting on lineage infidelity in pediatric b-cell acute lymphoblastic leukemia, or B-ALL. And joining me to discuss their findings are Dr. Kathrin Bernt, Dr. Fatemeh Alikarami, and Mr. Rushabh Mehta.

Dr. Bernt is a pediatric oncologist and an Associate Professor of Pediatrics at the Children's Hospital of Philadelphia. Dr. Bernt, welcome to the program.

Dr. Bernt:
It's a great pleasure being here.

Ryan Quigley:
And Dr. Alikarami is a Research Associate Scientist at the Children's Hospital of Philadelphia. Dr. Alikarami, thanks for being here as well.

Dr. Alikarami:
Thanks for having me.

Ryan Quigley:
And Mr. Mehta is a PhD candidate in cell and molecular biology at the University of Pennsylvania. Mr. Mehta, thank you very much for joining us.

Mr. Mehta:
Yeah, definitely. Thanks for having us on.

Ryan Quigley:
Of course. So to set the stage for us—and Dr. Bernt, this is for you—could you explain what lineage infidelity means in the context of B-ALL and why it's clinically significant?

Dr. Bernt:
So very broadly speaking, what we mean by lineage infidelity is that B-ALL cells may not be fully committed to a B-cell fate, and that can manifest in a variety of different ways. It can range from having just co-expression of markers of another lineage, classically of the myeloid lineage; it can manifest in what we call lineage drift or lineage shift to where, over the course of therapy, cells become less and less lymphoid and more and more myeloid; and it can also refer to subclonal heterogeneity, where within the same leukemia, we have cells that are more lymphoid or more myeloid within the same patient.

With respect to clinical significance, it varies by which of these different types of lineage infidelity we're dealing with, and it varies by subtype of leukemia. We've known for a long time that the expression of a myeloid marker here or there is quite common, and typically, it doesn't have any impact on outcome or treatment. But over the last several years, we've noticed an increasing rate in lineage drift and lineage shift, particularly under the pressure of B-cell directed immunotherapy and things like CAR T-cells or T-cell engagers that are directed against B-cell antigens. And for the most part, these are associated with exceptionally poor outcomes, really raising the question whether lineage infidelity underlies a lot of poor outcomes already and potentially even more so in the future.

On the flip side, we've defined a few subtypes of ALL where a lineage shift almost to a monocytic subtype is quite common early in therapy and doesn't seem to have much impact on outcome. Subtypes that do that are, for example, DUX-4 rearranged leukemias or

ZNF-584 rearranged leukemias. And in those, even though they can undergo a myeloid shift within the first couple of weeks, that really does not seem to impact outcomes too much. So there's a lot of open questions in this field and one of the reasons why we're excited about our study.

Ryan Quigley:
Thank you very much for that detailed breakdown, Dr. Bernt. And, Mr. Mehta, turning to you now, how did using a multiomic approach in this study help in identifying CD-19 negative subclones?

Mr. Mehta:
So in our study, using multiple modalities was essential to identifying the subclones, which were either CD-19 negative or even CD-19 dim. Initially, we looked at RNA to identify cells which resembled myeloid rather than lymphoid cells, and although we were pretty confident that these were leukemic because of their strong patient-specific signals, which you would expect with cancer cells, it was possible that they were just misannotated normal cells. So we were actually then able to use point mutation, so incorporating DNA, and that allowed us to confirm that these cells were leukemic because we were able to find point mutations which were either shared between lymphoid and myeloid blasts or specific to myeloid blasts. So once we incorporated the DNA, we were able to identify these cells as being leukemic.

Currently, we're validating our findings using both RNA and now incorporating protein into that as well. So this is through an approach which is known as CITE-seq, and we're able to identify myeloid-like cells, but the surface antigen expression, which we get through the protein, helps us confirm that they follow the traditional definition of myeloid cells too.

And then the last point that we want to incorporate here is that, because these patient samples come to us locally from CHOP, we have a lot of information like karyotyping, which we can then use to verify blast status as well. And this is done through inferring copy number variations in the RNA. So altogether, this study wouldn't have been possible had we not incorporated the different modalities.

Ryan Quigley:
For those just joining us, this is *Project Oncology* on ReachMD. I'm Ryan Quigley, and I'm speaking with Drs. Fatemeh Alikarami, Mr. Rashabh Mehta, and Dr. Kathrin Bernt about their research on lineage plasticity and therapy resistance in pediatric B-ALL.

Dr. Alikarami, this is for you now. I'd like to turn to the findings. One of the more striking outcomes was that chemotherapy seems to select for myeloid-like subclones. Can you walk us through how that works?

Dr. Alikarami:
So in general, chemotherapy can induce a phenotypic switch in leukemia by two main mechanisms. One is by eradicating the original clones, allowing a different pre-existing subclones to flourish. The next one is by altering the original clone, a differentiation program. In our work, our post-chemotherapy samples were collected after the end of induction. Usually, induction chemotherapy, specifically in B-ALL, uses a combination of chemotherapy drugs like steroids, prednisone, and vincristine, which are all highly effective against B-lymphoblastic leukemia cells, and they are much less effective against, myeloblasts.

So we speculate that this creates a survival advantage for preexisting CD-19 leukemia cells, that they are chemo-resistant, and they have a myeloid stem cell signature. And this selective pressure can contribute to residual disease that can evade detection and ultimately can contribute to relapse despite B-lymphoblastic leukemia cell clearance.

Ryan Quigley:
And, Mr. Mehta, the data also suggests that these subclones aren't restricted to KMT2A-rearranged B-ALL. How widespread is this across the disease spectrum?

Mr. Mehta:
So in our study, we profiled three high-risk subtypes outside of KMT2A-rearranged patients, so pH-like, pH-positive, and iAMP21—all high-risk subtypes. But while KMT2A patients have demonstrated the highest frequency of stem and myeloid cells, both at diagnosis and then post-treatment, we found that patients within all of our other profiled subtypes also demonstrated some degree of stem and myeloid subclones as well. And I think Kathrin mentioned this earlier, but there are other B-ALL subtypes that demonstrate these features at diagnosis.

But our finding is important because we see these blasts with improper lineage specification across non-KMT2A patients after the first month of chemotherapy, so distinctly different from what we see at diagnosis. We shouldn't generalize these findings to subtypes that we didn't profile here, but it will be important to determine whether these blasts with lower or absent expression of CD-19 are widespread in other subtypes as well.

Also, whether this is a feature solely of high-risk patients, who we specifically profiled in our study, or whether all patients demonstrate some level of lineage infidelity post-induction, remains to be answered as well.

Ryan Quigley:
Dr. Alikarami, back to you now. What are some of the clinical implications of these findings that you can foresee?

Dr. Alikarami:
I would focus on diagnostic implications. So the gold standard currently in US for monitoring MRD is standard flow cytometry using only traditional B-cell markers. Our findings strongly recommend that we should expand these monitoring panels by including stem cell/myeloid CD markers as well just because if we only rely on a standard flow cytometry using traditional B-cell markers, there is a high chance that we are missing these subclones that have a stem cell/myeloid signature at the first place, which could result in a misclassification of patient risk.

If there is a correlation between outcome and these myeloid stem cell subclones, I think it would be critical to capture them at the first place by adding these myeloid stem cell CD markers to the traditional panel for MRD detection.

Ryan Quigley:
And now, finally, before we wrap up this program—Dr. Bernt, this is for you now—if we look ahead, how might these findings influence future clinical trial design or risk stratification strategies in children with B-ALL?

Dr. Bernt:
Thank you. This is really the elephant in the room question. I think the first step we need to do is incorporate expanded diagnostics, exactly like Dr. Alikarami explained, into our upcoming clinical trials so that we clarify the key questions: how widespread is this phenomenon? Is this something that is only seen in high-risk ALL, or do we see this in standard-risk ALL as well? And does it have any therapeutic implications? Is the existence or is the degree to which we find either stem cell or myeloid-like clones linked to outcomes? I think we need this data before we really can take then the next step in discussing of how might that influence therapy.

On the flip side, I think it's pretty obvious that if you have an expanded myeloid clone that doesn't have CD-19 on the surface, then the addition of a T-cell engager that targets CD-19 would not be expected to work on that subclone. So if we indeed find that these clones are present in children who then go on to not respond well to T-cell engagers, to not respond well to continuing B-cell-type therapy, then the next step really is probably going to be to alter risk stratification based on such subclones to include other treatment modalities—either other immunotherapies that target different surface markers or more myeloid-style chemotherapy for those patients who don't respond. But again, too early for now. I think the critical part will be to analyze and track these clones through the upcoming next round of group-wide clinical trials.

Ryan Quigley:
And that's a great way to round out our conversation. And I want to thank my guests, Dr. Kathrin Bernt, Dr. Fatemeh Alikarami, and Mr. Rushabh Mehta for joining me to discuss these findings in pediatric B-ALL. Dr. Bernt, Dr. Alikarami, Mr. Mehta, it was great having you all on the program.

Dr. Bernt:
Thank you so much for having us.

Dr. Alikarami:
Thank you so much for having us.

Mr. Mehta:
Thanks, Ryan.

Ryan Quigley:
For ReachMD, I'm Ryan Quigley. To access this and other episodes in our series, visit *Project Oncology* on ReachMD.com, where you can Be Part of the Knowledge. Thanks for listening.