

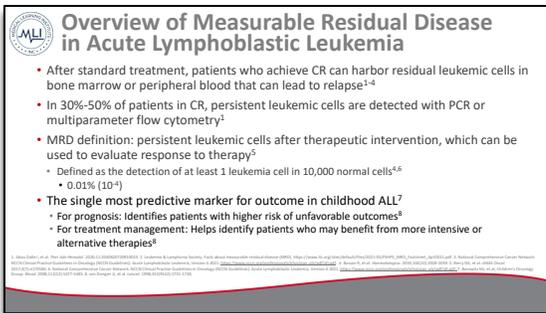
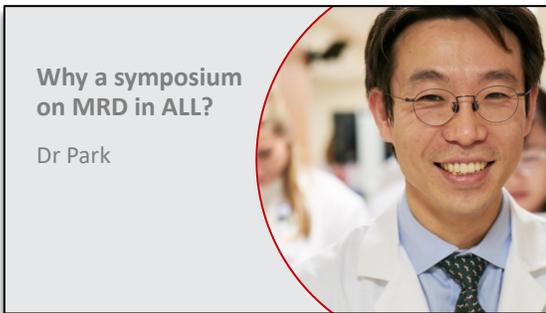


# MASTERING MRD FOR PROGNOSIS AND TREATMENT IN ALL

Originally presented at 2022 SOHO Annual Meeting on Friday, September 30, 2022



Dr. Park: Thank you for everyone to coming to this lunch symposium "Mastering MRD for Prognosis and Treatment in ALL." My name is Jae Park. I'm one of the leukemia physicians at Memorial Sloan Kettering Cancer Center, currently Acting Chief of the Cell Therapy Service and Director of Adult ALL program. I'm joined by my colleague, Dr. Rashmi Kanagal Shamanna, Associate Professor in the Department of Hematopathology and Director of Molecular Diagnostic Laboratory at the University of Texas, MD Anderson Cancer Center.



## Overview of Measurable Residual Disease in Acute Lymphoblastic Leukemia

I think a lot of you guys are all familiar with the role of MRD in ALL, but just to review. After standard therapy in ALL patients, the problem in ALL is not achieving complete remission as vast majority of the patients do achieve CR, which is defined by less than 5% bone marrow blast at the end of induction or at the end of consolidation. So either a 1-month mark, 3-month mark.

Most of our patients with ALL are going to be in complete remission.

However, about 30% to 50% of these patients still harbor residual leukemic cells below the 5% mark that we have arbitrarily set as a morphologic remission status. So, these residual cells can be detected either with PCR or multiparameter flow cytometry. MRD used to be called minimal residual disease, but the more modern name is measurable residual disease, and in that sense is really just indicating remaining disease burden at the level of the sensitivity.

In order to be called MRD, and this is important point for us to know, the limit of detection needs to be at least 1 leukemic cell in 10,000 normal cells or a sensitivity of 0.01% or  $10^{-4}$ . And importantly, the reason that we're talking about MRD is not only that we're leaving residual cells that after what we thought is complete remission, but that this is the single-most predictive marker for outcomes in childhood and adult ALL.

### Meta-Analysis Evaluating MRD in ALL<sup>1</sup>

- Meta-analysis of 39 studies pediatric and adult of patients (n=13,637) with ALL
- Prognostic significance of MRD clearance was demonstrated overall and in all subgroup analyses
  - Among therapies
  - MRD measurement method (PCR vs FCM)
  - MRD timing and cutoff value

10-year EFS and OS		
Pediatric Patients		
	MRD-negative	MRD-positive
EFS	77%	32%
OS	84%	55%
Adult Patients		
	MRD-negative	MRD-positive
EFS	64%	21%
OS	60%	15%

## Meta-Analysis Evaluating MRD in ALL<sup>1</sup>

Meta-analyses have looked at both children and adult patients with an ALL. And there's a clear demarcation of the patients who have MRD-positivity and MRD-negativity, both for event-free survival and overall survival.

So top panel is for patients, pediatric patients. There's a very clear separation by the MRD status. The MRD-negative patients do significantly better than MRD-positive. Bottom two panels are for adult patients. Again, this is showing the MRD-negative patients did significantly better than MRD-positive. But again, MRD-positive patients have significantly worse outcome. So, this is the reason that MRD is one of the better-known prognostic markers in ALL.

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### CALGB 10403 (AYA): Outcome by MRD Status

Of patients in CR1 at EO1, only 43% had undetectable MRD

## CALGB 10403 (AYA): Outcome by MRD Status

This is another study that was conducted. Again, the same pattern. The MRD-negative patients had a higher disease-free survival of 85% compared to 50% for those patients with an MRD-positive.

### Prognostic Value of MRD for Specific Treatment Types

- MRD status before HSCT predicts post-HSCT outcome<sup>1-4</sup>
- Positive MRD before transplant decreases PFS and OS and increases risk of relapse<sup>1-4</sup>
- Therapies that reduce MRD prior to HSCT may increase the likelihood of positive outcomes<sup>2</sup>
- In patients with negative MRD after HSCT or CAR T-cell therapy, a switch to MRD-positive status preceded relapse in all patients at 90 days and 60 days, respectively<sup>5</sup>
- One antibody treatment—blinatumomab—is indicated for MRD-positive patients<sup>6</sup>

## Prognostic Value of MRD for Specific Treatment Types

So not only at the end of induction or consolidation, MRD is very important for the prognostic information for both pediatric and adults with ALL. That MRD before the transplant, which we do note in some of the retrospective analysis, the MRD-positive prior to the time of transplant, those patients are more likely to relapse, which is another reason that, if we have an opportunity to do so, try to convert them into MRD-negativity before proceeding to transplant.

### Why Test MRD in Patients with ALL?

- Pediatric groups for ALL have been using MRD for risk stratification and therapeutic decision-making for years<sup>1,2</sup>
- MRD assessments are incorporated into treatment algorithms, including the NCCN guidelines for pediatric and adult patients<sup>3,4</sup>
  - \*MRD quantitation is an essential component of patient evaluation over the course of sequential ALL therapy<sup>3</sup>
- Commonly used as an endpoint in clinical trials to monitor disease; every clinical trial in ALL assesses MRD<sup>5</sup>
- Blinatumomab is prescribed based on MRD positivity, validating the importance of MRD as an endpoint<sup>6,7</sup>

## Why Test MRD in Patients with ALL?

So hopefully this gave you some idea of why we should be testing MRD in patients with ALL. Again, it's now a prognostically very important landmark. And because of that, several guidelines, including the NCCN guidelines, do suggest and recommend MRD testing. And we'll be talking about when to test for MRD and how to test for MRD as we're talking now about why we're testing for the MRD.

There are many clinical trials in ALL, and I would argue that all clinical trials now being conducted in ALL, include looking at MRD, whether as a primary endpoint or the surrogate endpoint, or the secondary or the exploratory endpoint. And we do now have a therapy that's specifically approved for MRD indications, so

there's another reasons that not only helps us to figure out who's at high risk of relapse, but we can actually do something to convert their risk.

**Variability in MRD Testing**

- Lack of gold standard technique and lack of understanding of timing as described in NCCN guidelines increases variability in MRD testing across clinical practices<sup>1</sup>
- Within clinical trials, variation exists in the timing, analysis method, and sensitivity of MRD testing<sup>2</sup>
- In real-world ALL disease management, there are differences in MRD testing between patient groups and practice settings<sup>3</sup>
  - Adult patients: 73%; pediatric patients: 93%
  - Community practices: 67%; academic centers: 84%

### Variability in MRD Testing

So, one of the challenges is the variability in MRD testing, and we're going to be spending a great amount of the time in the second part of the talk by Dr. Kanagal Shamanna about two different assays and then how to interpret the result of those.

**Poll**

How often do you measure MRD in patients with ALL?

- A. Never
- B. Rarely
- C. On a case-by-case basis
- D. Frequently
- E. Always

### Poll

All right, the first poll. How often do you measure MRD in patients with ALL? Never, rarely, case by case, frequently, or always?

I'll argue that we should all be testing for MRD always, and the vast majority of you guys are testing for the MRD always, which we like.

**Timing of MRD Testing**

Dr Park



**How Does MRD Change Over the Course of ALL?**

**Case Study:** A 67-year-old woman with Ph negative B-ALL

- Started induction with miniCVD + inotuzumab
- After cycle 1A (ie, 4 weeks after initial therapy), she achieved MRD-negative CR as assessed by multiparameter flow cytometry with at least  $10^{-4}$  sensitivity
- She continued on the regimen and completed all 8 cycles of this multiagent chemotherapy with inotuzumab
- She had bone marrow assessment including MRD after cycle 2A and 3A and after 4B (ie, after all consolidation chemotherapy)
- She then proceeded to POMP maintenance chemotherapy; she tolerated the treatment well with no symptoms
- At 3 months of maintenance, she had a routine follow-up bone marrow assessment, which showed a detectable MRD at 0.1%

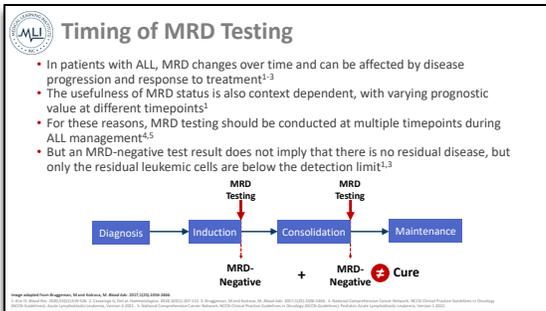
### How Does MRD Change Over the Course of ALL?

Regarding the timing of MRD, I'm going to start with a case. This is mimicking a case that I recently saw in clinic. So, a 67-year-old woman with a Ph-negative B-cell ALL. But given her age, she received induction with miniCVD or a dose-reduced intensity to chemotherapy with inotuzumab combined. After cycle 1A, which is essentially about four weeks after initial therapy, she achieved MRD-negative CR as assessed by the

multiparameter flow cytometry, which is the assay that we are using at my institution with at least  $10^{-4}$  sensitivity.

She continued on the regimen and completed all eight cycles of. And she had a bone marrow assessment during those cycles, after cycle 2A, which is a three-month mark, and after cycle 3A, and after 4B. So, she did have frequent bone marrows checking for MRD at each timepoint, and she was negative for all.

Because of that, she ended up proceeding to maintenance therapy with POMP. She tolerated treatment well, and then we performed a three-month bone marrow biopsy after study maintenance, and that bone marrow unexpectedly showed an MRD level of 0.1%, even though she had a completely normal CBC.



### Timing of MRD Testing

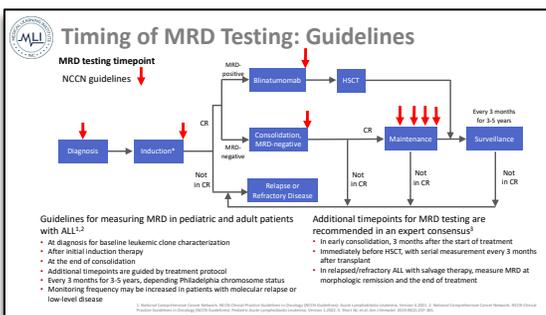
So that brings the point of the timing of testing for MRD. I think the important point here is that MRD is not a single timepoint assessment. It's a dynamic process. So, you definitely want to get them after initial induction. That's probably the first timepoint that most of us are assessing for MRD. In the pediatric protocols, some actually mandate MRD assessment at week 2. A lot of adult protocols, it would be about week 4. And

depending on the regimen in adult ALL, after induction could mean 4 weeks of a therapy or 12 weeks of a therapy. And it's another challenging part when we were building the NCCN guidelines to exactly specify what timepoints because induction on different regimens for ALL could mean different timepoints.

But in general, by 3 months, they really should have at least one MRD assessment. They should be tested throughout the consolidation because consolidation is often about six to eight months of therapy. So, these patients would be getting serial bone marrow biopsies; and every time they're getting the bone marrow biopsies, you should also be testing for the MRD. And then before maintenance, also we did the MRD testing; and during maintenance you should also be checking for the MRD afterwards too. So, it is a dynamic process, not a single timepoint, although single test does give us prognostic information. But just because they are MRD-negative once, that doesn't mean they're going to continue to stay MRD-negative. So that's the reason that it's important to test again.

And then the second point is the goal of an ALL therapy for the vast majority of the patients is cure, and that's where the MRD assessment becomes even more important because we may need to intensify the therapy to achieve that goal.

And MRD-negativity, even at single timepoint or even at dynamic timepoint during the first year or so, doesn't mean a cure. It is a first step to cure, but MRD-negativity doesn't guarantee cure. So, you have to continue to monitor these patients afterwards.



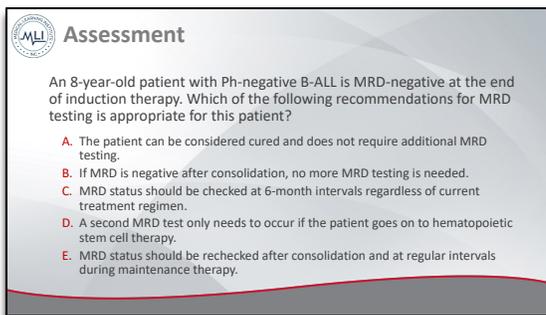
### Timing of MRD Testing: Guidelines

So, the NCCN guidelines have some wording to guide our clinicians who may not see ALL patients as often. But I have to admit that it is a little vague for the reasons I mentioned before. So, at the end of induction, consolidation, and during maintenance. But the important point is it's a dynamic process. We need to continue to monitor through each one.

And then the treatment varies based on your MRD assessment. So, if you are CR but MRD-positive, these patients will be receiving blinatumomab, which is the medicine that's approved for MRD indication. After the treatment, they should be testing to see whether they're still MRD-positive or not. And then we have to

make a decision whether to go transplant even with MRD-positivity or try another therapy to convert that into MRD-negative before going to transplant. If those patients are MRD-negative after induction of consolidation, they continue with the current regimen. The arrows here indicate the continued assessment of the MRD as needed.

In relapsed-refractory disease, MRD assessment, the value of that is not as clearly established. But I would argue too that if these patients are also going to transplant, and especially for the B-cell ALL patients, whether we have a little bit more data and tools to convert them into MRD-positivity, MRD-negative, even in the relapsed/refractory patients before these patients go to transplant or after their salvage chemotherapy regimen or salvage regimens, MRD should be assessed.



**Assessment**

An 8-year-old patient with Ph-negative B-ALL is MRD-negative at the end of induction therapy. Which of the following recommendations for MRD testing is appropriate for this patient?

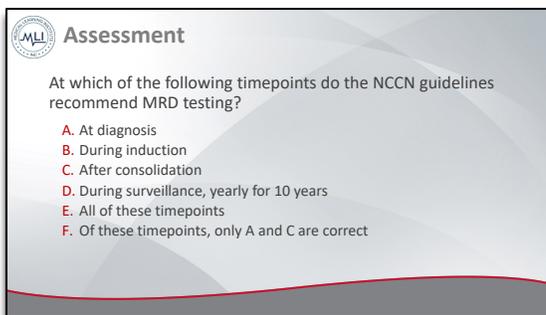
- A. The patient can be considered cured and does not require additional MRD testing.
- B. If MRD is negative after consolidation, no more MRD testing is needed.
- C. MRD status should be checked at 6-month intervals regardless of current treatment regimen.
- D. A second MRD test only needs to occur if the patient goes on to hematopoietic stem cell therapy.
- E. MRD status should be rechecked after consolidation and at regular intervals during maintenance therapy.

### Assessment

So, an 80-year-old patient with a Ph-negative B-ALL is MRD-negative at the end of induction therapy. Which of the following recommendations for MRD testing is appropriate for this patient? The patient can be considered cured and does not require additional MRD testing after induction. B) If the MRD is negative after consolidation, no more MRD testing is needed. C) MRD status should be checked at six-month intervals, regardless of the current treatment regimen. D) A second MRD

testing only needs to occur if the patient goes to hematopoietic stem cell therapy. And the last, E) MRD status should be rechecked after a consolidation and at regular intervals during maintenance therapy.

The current recommendation is for the MRD testing to be checked after consolidation and regular intervals as we talked about.



**Assessment**

At which of the following timepoints do the NCCN guidelines recommend MRD testing?

- A. At diagnosis
- B. During induction
- C. After consolidation
- D. During surveillance, yearly for 10 years
- E. All of these timepoints
- F. Of these timepoints, only A and C are correct

### Assessment

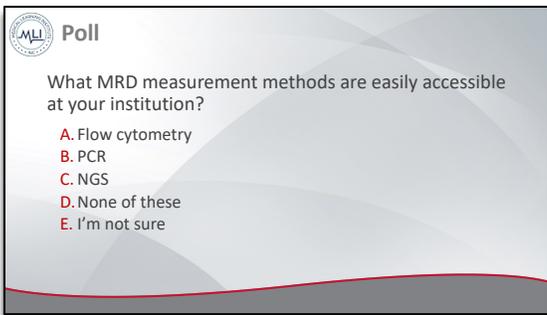
Which of the following timepoints do the NCCN guidelines recommend for MRD testing? At diagnosis, during induction, after consolidation, surveillance yearly for 10 years, all of these, or of these timepoints, only A and C, which is a diagnosis after consolidation is correct?

It was not mean to be tricky, but I realize why it could be. All right, so the correct answer here was A and C. The thing was that for B, it's during induction. So even though I think you

could check during induction, although they were not recommending that in the midst of therapy. But some pediatric protocols include week 2 MRD assessment. But for the vast majority of adult ALL studies, they do not. So, I think that was kind of the one, the one caveat. So, at diagnosis, at consolidation it's a minimal timepoint, again, during the maintenance dynamic process.



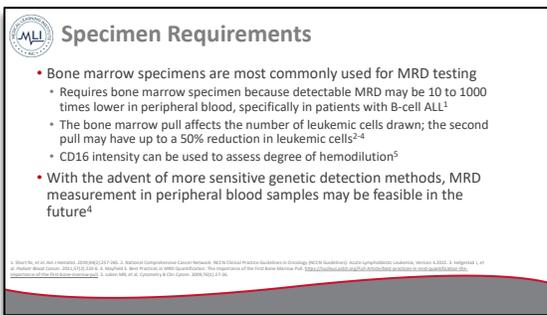
**Dr. Kanagal Shamanna:** So, thank you, Dr. Park. The aspects that I'm going to be touching on would be the perspective of a pathologist or a testing laboratory.



### Poll

So first, I would like to take a poll. What MRD measurement methods are easily accessible at your institution? So, A) Flow cytometry. PCR, NGS, none of these.

So, the majority voted for flow cytometry.



### Specimen Requirements

So as a laboratory professional, I think Dr. Park has emphasized enough the importance of MRD testing, that it is the single-most predictive biomarker for assessment of outcomes. So, what does that mean? It means that in terms of testing, we need to be consistent, and there needs to be a standardization across every lab that is in the US and outside as well.

For example, if a patient comes to my lab for testing, and I call it MRD-positive, and the patient with the same sample is tested at say Sloan Kettering, both should provide the same results. So, in terms of laboratory, we always look at three parameters: the preanalytical, the analytical is actual testing, and the postanalytical. So, rest of my talk will flow along those lines.

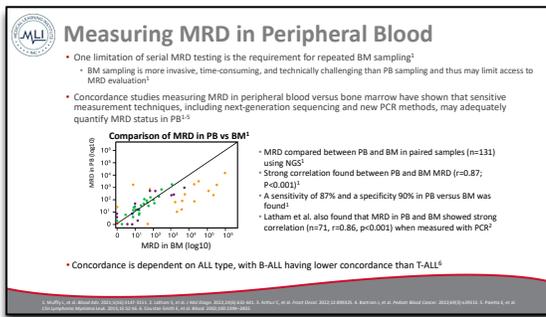
So preanalytical, the most important parameter would be the sample type. So, for the MRD testing, right now the standard recommendation would be the bone marrow specimen. This can be quite challenging, especially for pediatrics. But based on the sensitivity of the flow cytometry, which is the most frequent as you all voted, technology, it is still recommended to do bone marrow aspirates. However, there are studies out there that are looking into comparison between bone marrow and peripheral blood samples. Well, it matters because in the peripheral blood, the tumor burden is nearly 10 to 1,000 times lower, which means that the flow cytometry that has an LOD of about  $10^{-4}$  may not pick up anything lower than that and might give a false-negative result.

In addition to the type of specimen, the pull of the aspirate matters. So, when we were doing the bone marrow aspiration, the very first concentrated aspirate specimen, about 2.5 milliliters to 3 milliliters, this is all the marrow that is actually representative of what's in the patient. Anything after 3 milliliters is actually mixed in with the peripheral blood that's bleeding into the sample.

So, in fact, the NCCN recommends the first full sample to be used for MRD because that is the most accurate representation of the residual disease in the patient. Of course, laboratory-wise, we don't know what is coming in. Sometimes there might be an indication of the number of the pull, but majority of the times we don't. So, we do have a way to evaluate the degree of hemodilution in some of these samples using the CD16 intensity.

So, the CD16 is super bright in mature neutrophils. So, if there's a lot of CD16 bright-positive cells, it is a hint that it might be a hemodiluted sample. Why is that important? It's important for us to notify the oncologist that the report we are giving out may not have come from representative samples, and it's important to put together the entire clinical findings and everything else and maybe monitor the patient more closely and repeat the marrow as needed.

The good thing is that now with the advent of more sensitive assays, molecular-based assays, including NGS, the peripheral blood could potentially be useful because the sensitivity goes down to  $10^{-5}$  or even  $10^{-6}$ . So, this would be extremely beneficial to pediatric patients because it's an invasive procedure all said and done.



**Measuring MRD in Peripheral Blood**

- One limitation of serial MRD testing is the requirement for repeated BM sampling<sup>1</sup>
  - BM sampling is more invasive, time-consuming, and technically challenging than PB sampling and thus may limit access to MRD evaluation<sup>2</sup>
- Concordance studies measuring MRD in peripheral blood versus bone marrow have shown that sensitive measurement techniques, including next-generation sequencing and new PCR methods, may adequately quantify MRD status in PB<sup>3,4</sup>

**Comparison of MRD in PB vs BM<sup>5</sup>**

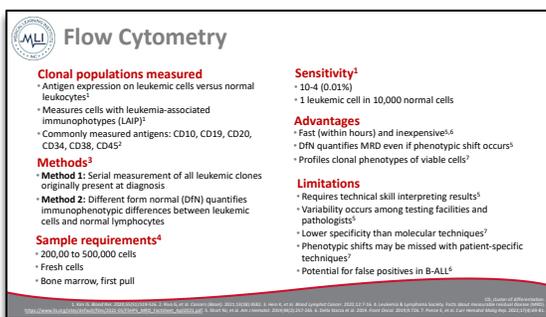
MRD in PB (log10) vs MRD in BM (log10)

- MRD compared between PB and BM in paired samples (n=131) using NGS<sup>5</sup>
- Strong correlation found between PB and BM MRD ( $r=0.87$ ;  $P<0.001$ )<sup>5</sup>
- A sensitivity of 87% and a specificity 90% in PB versus BM was found<sup>5</sup>
- Latham et al. also found that MRD in PB and BM showed strong correlation (n=71,  $r=0.86$ ,  $p<0.001$ ) when measured with PCR<sup>6</sup>

Concordance is dependent on ALL type, with B-ALL having lower concordance than T-ALL<sup>6</sup>

### Measuring MRD in Peripheral Blood

One example of a very large study that looked into comparing the peripheral blood and bone marrow in nearly 131 patient samples found a very strong correlation. And this, of course, was based on the NGS method of assessment.



**Flow Cytometry**

**Clonal populations measured**

- Antigen expression on leukemic cells versus normal leukocytes<sup>1</sup>
- Measures cells with leukemia-associated immunophenotypes (LAIP)<sup>2</sup>
- Commonly measured antigens: CD10, CD19, CD20, CD34, CD38, CD45<sup>3</sup>

**Methods<sup>3</sup>**

- Method 1:** Serial measurement of all leukemic clones originally present at diagnosis
- Method 2:** Different from normal (DN) quantifies immunophenotypic differences between leukemic cells and normal lymphocytes

**Sample requirements<sup>4</sup>**

- 200,000 to 500,000 cells
- Fresh cells
- Bone marrow, first pull

**Sensitivity<sup>1</sup>**

- $10^{-4}$  (0.01%)
- 1 leukemic cell in 10,000 normal cells

**Advantages**

- Fast (within hours) and inexpensive<sup>1,4</sup>
- DN quantifies MRD even if phenotypic shift occurs<sup>5</sup>
- Profiles clonal phenotypes of viable cells<sup>5</sup>

**Limitations**

- Requires technical skill interpreting results<sup>5</sup>
- Variability occurs among testing facilities and pathologists<sup>5</sup>
- Lower specificity than molecular techniques<sup>5</sup>
- Phenotypic shifts may be missed with patient-specific techniques<sup>5</sup>
- Potential for false positives in B-ALL<sup>6</sup>

### Flow Cytometry

Then moving into the analytical component is the testing methodology. There are several methods of available. So, flow cytometry, the molecular methods of qPCR, which is a pretty standard technology used in the European continent, and then we have the NGS. The wave is coming in, and it's here to stay from the data.

So, let's look at each of these technologies, the pros and cons, and then it's important as treating physicians to be aware of this and to deal with the specimens to modify the ordering patterns as needed.

So, the flow cytometry is based on the antigen expression on the leukemia cells compared to the normal bone marrow mononuclear cells. So, it measures the cells with leukemia-associated immunophenotypes using a variety of antibodies.

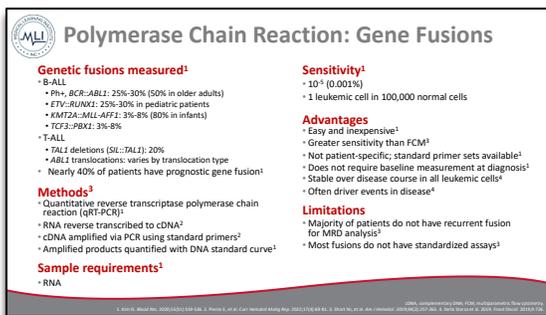
There is also a second method called "different from normal" where it quantifies the immunophenotypic differences between the leukemia cells and the normal phenotype. So, if you use a different from normal combined with the leukemia-associated immunophenotype, the interpretation is more accurate.

The sample requirements would be at least 200,000 to 500,000 cells just because you need that many cells to identify the leukemia positivity, the residual disease positivity at the proposed LOD. It's important to get the sample fresh, but it can be up to 48 hours old. Anything older, a viability assay needs to be performed. And of course, as I mentioned before, the first pull from the bone marrow is recommended. It is recommended by the NCCN that the sensitivity of the flow panel used at any laboratory, which varies even within the US, is at least  $10^{-4}$ , which can detect 1 leukemia cell in 10,000 normal cells.

Now what are the advantages? It's fast. You're getting the sample fresh. The result is available within a few hours. It's relatively inexpensive compared to NGS. And in some cases, during relapses or even at the time of MRD, if the immunophenotype shifts, the different from normal technique can still be used to quantify the MRD. And, of course, you are getting the phenotype of the cells that are viable because you're removing all the debris.

Now the main limitation is that it requires technical skill, a trained pathologist to perform the interpretation, and this could be variable across different labs. And there needs to be a consensus guideline standardizing the panel, the interpretation, the report, etc.

And, of course, during immunophenotypic, if there are large immunophenotypic shifts, an untrained eye may fail to see these minute populations. And, of course, there's a potential of false-positives, especially in B-ALL, especially because of the hematogones.



**Polymerase Chain Reaction: Gene Fusions**

**Genetic fusions measured<sup>1</sup>**

- B-ALL
  - Ph+, BCR-ABL1: 25%-30% (50% in older adults)
  - ETV6-RUNX1: 25%-30% in pediatric patients
  - KMT2A-MLL-AFF1: 3%-8% (80% in infants)
  - TCF3-PBX1: 3%-6%
- T-ALL
  - TAL1 deletions (5L::TAL1): 20%
  - ABL1 translocations: varies by translocation type
  - Nearly 40% of patients have prognostic gene fusion<sup>2</sup>

**Methods<sup>3</sup>**

- Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)<sup>1</sup>
- RNA reverse transcribed to cDNA<sup>2</sup>
- cDNA amplified via PCR using standard primers<sup>2</sup>
- Amplified products quantified with DNA standard curve<sup>2</sup>

**Sample requirements<sup>1</sup>**

- RNA

**Sensitivity<sup>1</sup>**

- $10^{-5}$  (0.001%)
- 1 leukemic cell in 100,000 normal cells

**Advantages**

- Easy and inexpensive<sup>1</sup>
- Greater sensitivity than FCM<sup>1</sup>
- Not patient-specific; standard primer sets available<sup>1</sup>
- Does not require baseline measurement at diagnosis<sup>1</sup>
- Stable over disease course in all leukemic cells<sup>1</sup>
- Often driver events in disease<sup>4</sup>

**Limitations**

- Majority of patients do not have recurrent fusion for MRD analysis<sup>1</sup>
- Most fusions do not have standardized assays<sup>1</sup>

## Polymerase Chain Reaction: Gene Fusions

So, the molecular methods, the first, the most convenient would be ALL cases with a known translocation. Nearly 40% of ALL cases have some sort of a standard translocation. So, in B-ALL would be the Ph+, ETV6-RUNX1, KMT2A arrangements, etc. The T-ALLs also have a few translocations to ABL1 fusions, TAL1 translocations and deletions.

A convenient way to measure that would be using the reverse transcriptase PCR from the RNA. Now remember these translocations can have different breakpoints. So, if we tried to do a DNA-based assay, we need extensive primers to pick up all possible translocations. On the other hand, if you use an RNA product and then reverse transcribe it to cDNA and then evaluate, the yield is much higher.

The sample requirements is, of course, you just need RNA. You don't need fresh cells as long as the RNA has been extracted and preserved properly.

The sensitivity is much higher than the flow. It is  $10^{-5}$ . It is relatively easy to perform, inexpensive, more sensitive than flow. It is not patient specific. We can use standard set of primers to evaluate for translocations. And many of these have already been standardized for chronic myeloid leukemia, etc. So, they're readily available in most labs. The most important thing is these fusions are drivers of the diseases, so it really helps to characterize them. The limitation is that 60% of cases don't have a fusion.

**PCR: Gene Translocations**

**Genetic translocations measured<sup>1</sup>**

- Used in Ph-negative ALL
- Unique sequences in rearranged IGH or TCR genes

**Methods**

- Allele-specific oligonucleotide PCR (ASO-PCR)<sup>2</sup>
- Identify patient-specific primers for serial testing<sup>2</sup>
- Establish amplification conditions and sensitivity for each ASO<sup>2</sup>

**Sample requirements<sup>1</sup>**

- DNA<sup>1</sup>

**Sensitivity<sup>1</sup>**

- $10^4$  (0.001%)
- 1 leukemic cell in 100,000 normal cells

**Advantages**

- Extensively standardized within the EuroMRD Consortium<sup>1</sup>
- Cross-lineage rearrangements are common<sup>2</sup>
- 90% of B-ALL can express T-cell receptor<sup>2</sup>
- 20% of T-ALL show IGH rearrangements<sup>2</sup>
- 90%-95% of patients have quantifiable rearrangements<sup>4</sup>

**Limitations**

- Time-consuming and technically complex<sup>4</sup>
- Needs baseline assessment and ample DNA<sup>1</sup>
- 5%-10% of patients with ALL do not carry IGH/TCR gene rearrangements<sup>2</sup>
- Difficult to monitor in "immature" immunophenotype ALL<sup>4</sup>
- Clonal evolution can lead to false negatives<sup>2</sup>
- Not able to assess "positive-not-quantifiable" (PNQ) results<sup>2</sup>
- Non-specific amplification can lead to false positives<sup>2</sup>

## PCR: Gene Translocations

The PCR is to look for gene rearrangements of the immunoglobulin sequence using a qPCR assay. This is a pretty standard method to identify MRD in the European continent. It's called the allele-specific oligonucleotide PCR. The one caveat with that is you need to know the full profile of the baseline sample, and it needs to be patient specific. So, each patient would have his or her own set of primers that the laboratory has

to generate at every MRD timepoint in order to follow that sequence.

The DNA sample requirement is easier than RNA even. Sensitivity is still  $10^5$ . It's been extensively standardized by the EuroMRD Consortium. And the one advantage is some of the B-ALLs can have the cross-lineage rearrangement, so you can have a T-cell rearrangement clone in B-ALL and vice versa.

And I mentioned the limitations would be that it's time consuming, it's technically complex, and it, obviously, needs a baseline profile. And of course, rarely, some cases may not carry the immunoglobulin rearrangement, and these might be difficult to assess.

**Next-Generation Sequencing**

**Genetic alterations measured<sup>1</sup>**

- Rearrangements in IGH/TCR genes

**Methods**

- PCR amplification of target DNA sequences<sup>2</sup>
- Parallel DNA sequencing allows for simultaneous detection of many gene rearrangements<sup>2</sup>
- Uses universal primers; not patient-specific sequences<sup>1,3</sup>
- Ability to track minor subclones<sup>1</sup>
- ClonoSEQ approved by FDA for MRD detection in ALL<sup>4</sup>
- Requires pre-treatment analysis

**Sample requirements**

- DNA<sup>1</sup>

**Sensitivity<sup>2,5</sup>**

- $10^5$  (0.0001%); potentially to  $10^7$
- 1 leukemic cell in 1,000,000 normal cells

**Advantages**

- Relatively fast: ~1 week for results<sup>6</sup>
- High concordance with standard MFC or PCR<sup>1,8</sup>
- High sensitivity may result in better prediction of poor and excellent outcomes<sup>1,8</sup>
- Simultaneous detection of all IGH/TCR<sup>1</sup> rearrangements<sup>3</sup>
- Reduction of "positive not quantifiable" samples<sup>10</sup>
- High level of sensitivity useful for MRD detection in peripheral blood<sup>1</sup>
- Potential detection of early evolution of clones during relapse<sup>3</sup>
- Excellent standardization in Europe<sup>1</sup>

**Limitations**

- Technically complex<sup>6</sup>
- May be prohibitively expensive<sup>2</sup>
- Not currently standardized in United States<sup>2</sup>
- Many cells required to achieve highest level of sensitivity<sup>3</sup>

## Next-Generation Sequencing

The final method that I'm going to touch upon is next-generation sequencing. Here you are measuring not only one set of clonal index sequence, you're measuring all possible index sequences in that patient sample, including the normal cells. So, it allows for characterization of the entire immune repertoire so to speak. So, you can identify single clones or there might be multiple clones. It uses a universal set of primers, no patient-specific

primers, so it's easy for the laboratory to manage. And you can even track minor subclones or even new clones that might emerge during relapse.

The ClonoSEQ happens to be the first FDA-approved NGS-based technique for MRD detection. And many, many of the labs are moving towards that.

And of course, it absolutely requires baseline profiling to know which clone to follow. If you do not have the baseline, it's almost impossible to do subsequent analysis. You require DNA sensitivity  $10^6$ , so it's the highest degree of sensitivity, which is why peripheral blood samples are extremely useful for this type of analysis and comparable to bone marrow too. And I think it takes a one-week turnaround time, which is relatively okay. It's not unacceptable for management.

There is a high concordance many studies have shown between flow cytometry and other techniques. And one important thing is the normal immune repertoire. There are studies that have shown that the lower degree of immune repertoire also has a worse outcome. So, there are several other parameters that are not explored much that could come into play. This is, of course, technically complex and only a really complex laboratory can assess for these.

### Comparison of MRD by Flow Cytometry and High Throughput Sequencing

- Paired pretreatment and EOI (day 29) samples from 619 patients enrolled in two Children's Oncology Group studies were evaluated using MFC and NGS<sup>1</sup>
  - AALL0331 (standard risk protocol, n=304)
  - AALL0232 (high-risk protocol, n=315)
- IGH and TRG CDR3 regions were amplified and sequenced

- MRD status showed similar 5-year EFS and OS in HTS and FC analyses
- 55 patients with FC MRD-/NGS MRD+ showed inferior 5-year EFS
- NGS was able to identify higher-risk patients within the SR group

## Comparison of MRD by Flow Cytometry and High Throughput Sequencing

I think I already touched upon this study highlighting how the flow cytometry and the high throughput sequencing using NGS compared to each other. MRD positivity by either of the techniques, as shown in this study, predicted poor survival. But importantly, about 55 patients who were negative by flow did show MRD-positivity and they had an inferior overall survival. This was done in pediatrics.

### MRD Assessment in ALL Using NGS is More Sensitive Than Flow Cytometry

- BM samples from 74 adults were analyzed with MFC and NGS
- ~46% of flow-negative cases were positive by NGS-MRD
- NGS was a better predictor for relapse than MFC

## MRD Assessment in ALL Using NGS is More Sensitive Than Flow Cytometry

So, this is another recent study from MD Anderson in adult patients showing similar data. This was in a 74-patient series, and 46% of flow-negative MRD cases were positive by NGS. The conclusion was that NGS was a better predictor for relapse than flow cytometry.

### Comparison of MRD Assays<sup>1,2</sup>

MRD Method	Sensitivity	Advantages	Disadvantages
Multiparameter Flow Cytometry (FCM)	10 <sup>-4</sup> (0.01%)	<ul style="list-style-type: none"> <li>Fast</li> <li>Cost effective</li> <li>Widely available platform</li> <li>Clinically proven platform</li> </ul>	<ul style="list-style-type: none"> <li>Subjective interpretation</li> <li>Immunophenotype may change during treatment</li> <li>Inadequate standardization</li> <li>Immunotherapy treatment can complicate interpretation</li> </ul>
RQ-PCR to Igh/TCR gene rearrangements	10 <sup>-4</sup> - 10 <sup>-5</sup> (0.01%-0.001%)	<ul style="list-style-type: none"> <li>Well-standardized</li> <li>More sensitive than FCM</li> </ul>	<ul style="list-style-type: none"> <li>Technically labor intensive</li> <li>Requires technical expertise</li> <li>Expensive</li> </ul>
qRT-PCR for gene fusions	10 <sup>-4</sup> - 10 <sup>-5</sup> (0.01%-0.001%)	<ul style="list-style-type: none"> <li>More sensitive than FCM</li> <li>Technically simpler</li> </ul>	<ul style="list-style-type: none"> <li>Need for baseline specimen</li> <li>Limited standardization</li> <li>Not all ALL cases have a gene rearrangement - immature T-ALL</li> </ul>
Next generation sequencing	10 <sup>-6</sup> (0.0001%)	<ul style="list-style-type: none"> <li>Very sensitive</li> <li>Relatively fast</li> </ul>	<ul style="list-style-type: none"> <li>Not standardized yet</li> <li>Requires bioinformatics</li> <li>Limited clinical validation</li> <li>Expensive</li> </ul>

## Comparison of MRD Assays

Here is a summary to go over the different techniques [Handout]

### Factors to Consider When Determining MRD Measurement Technique

- What laboratory facilities are available at my institution/clinic?
- How quickly do I need the MRD test results?
- Do I have enough sample for the technique?
  - "What pull is the sample"
- What does the morphology show?
- How old is the sample?
- What were the baseline characteristics? Did the sample have BCR::ABL1?
  - if so, RT-PCR would be the best method for MRD measurement.
- How much sensitivity do I need in the MRD measurement?
- What specimen type do I have? Bone marrow? Blood?
- How many times does MRD need to be measured?
- Do I have access to centralized lab or standardized assay?
- What technical and interpretation expertise is available?

## Factors to Consider When Determining MRD Measurement Technique

And the factors to consider when you want to determine which MRD to use and which laboratory to send the samples to.

### Assessment

Which of the following MRD techniques needs baseline data from a diagnostic specimen?

- RT-PCR for BCR::ABL1 fusion transcript
- NGS assay for antigen rearrangement
- Flow cytometry
- None of the above
- All of the techniques need baseline data

## Assessment

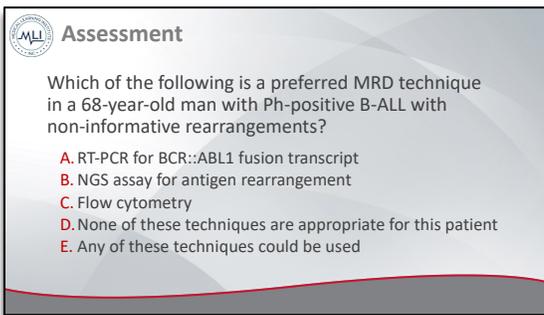
Which of the following MRD techniques needs baseline data from a diagnostic sample? RT-PCR for BCR-ABL fusion, NGS assay for antigen rearrangement, flow cytometry, none of the above, all of the techniques need baseline data?

The answer would be the NGS assay. Ideally it would help to know the baseline data for every technique to make it easier

and more accurate. If we know that the baseline is possible, if you happen to do another PCR assay for BCR-ABL and it turns out positive, you really do not need to know the baseline data. The cytogenetics could have been informative at that time. So, it's not absolutely needed.

For flow cytometry, we are a referral institution and often we get samples for interpretation at the time of MRD, so we really don't have the baseline data. But the difference from normal technique that I highlighted before, and I'm going to show in a subsequent slide, we can evaluate for immunophenotypic differences and really characterize them.

It is tough. We might not be 100% accurate. We could suggest either way, but it's not absolutely needed. But for NGS, it's impossible to evaluate without the baseline clone.



**Assessment**

Which of the following is a preferred MRD technique in a 68-year-old man with Ph-positive B-ALL with non-informative rearrangements?

- A. RT-PCR for BCR::ABL1 fusion transcript
- B. NGS assay for antigen rearrangement
- C. Flow cytometry
- D. None of these techniques are appropriate for this patient
- E. Any of these techniques could be used

Assessment

Which of the following is a preferred MRD technique is a, in a 68-year-old man with Ph-positive B-ALL with noninformative rearrangements? I think this is pretty obvious, Ph-positive, so BCR-ABL would be pretty accurate.



Q&A

So, Q&A, open to questions. How do you deal with discrepancies between flow and qPCR NGS data in Ph-positive ALL? Do you look for myeloid cell, BCR-ABL expression?

We do not actually. Based on the studies out there, any discrepancy between flow and the NGS data, we believe the NGS data would be more accurate than the flow. And we do not sort for the myeloid cells to look for BCR-ABL expression. But

there is a way by FISH where you can actually go back to your FISH and try to map out the cell that is staining for the fusion product. But like I said, the NGS is way more sensitive. And if it's performed in a standardized laboratory, we take that as the more accurate.

**Speaker:** Thank you for the excellent presentation. Two questions. The first question is for a patient who has clone-defining disease like Philadelphia-positive. So, some of these patients maybe have another clone that can identify the disease. So, if you choose the Philadelphia chromosome to identify it, then maybe it's not the only way to assess the MRD. Maybe you can comment on that.

The second question, a patient received direct therapy for some of the clone that has phenotype and, by flow cytometry like CD19-like CAR T-cell therapy. So how do you want to assess that clone with the directed therapy? Thank you.

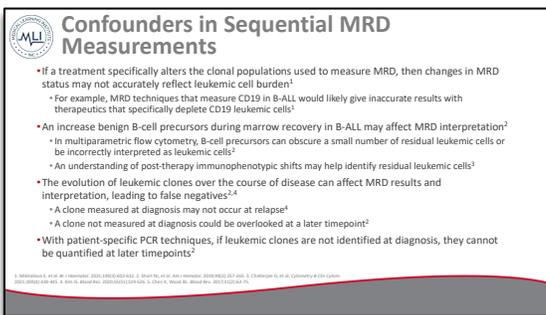
**Dr. Kanagal Shamanna:** Yeah, for the first question I absolutely agree it would be ideal to do the NGS-based technique as well as RT-PCR for the reason that you mentioned. But of course, we also have to look

at the resources, the patient insurance, whether they're going to pay for it, and balance the cost. But, ideally, yes, both of them.

The second CD19 CAR, I think it would be helpful for the oncologists to put a note for us to start off with the interpretation, and I think the information we get from the oncologists, or the ordering provider is critical for the subsequent way we process the samples. If you are using a CD19 gate, for example, we should be aware of the prior therapy, absolutely. Thank you for bringing that point.



Dr. Kanagal Shamanna: So interpreting results of MRD testing.



### Confounders in Sequential MRD Measurements

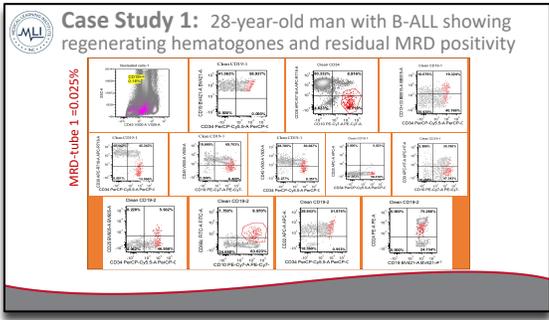
So, as I mentioned before, the interpretation can be pretty complex, both with respect to flow cytometry and NGS, and needs a significant degree of expertise, especially in B-ALL. If in younger patients, for example, there might be an exaggerated response from the hematogones, regenerating hematogones that can occur after chemotherapy, or stem cell transplant, or if the patient has a concurrent inflammatory disorder. All of that could

confound interpretation.

Secondly, if the treatment alters the population, like you brought up the CAR-T therapy targeting the CD19, that could be a definite confounder for the interpretation.

Thirdly, the evolution of leukemic clones could change. There might be certain immunophenotypic shifts at the time of relapse or the flow cytometry MRD level relapse. And it's important for the interpreter to put all the data together to formulate the interpretation.

And fourthly, especially with the PCR techniques, if we are not aware whether it was positive at the time of testing, and we only had the MRD sample, the PCR primers cannot cover all the breakpoints. Suppose we do know the BCR-ABL was positive, and that particular breakpoint is not covered by the BCR-ABL PCR assay. And if you're not aware that it was detected by PCR at the time of baseline, it could cause a false-negative interpretation.



### Case Study 1:

So, by far the most important or challenging would be the regenerating hematogones. Although I'm not going to go over the complexity here due to lack of time, I do want to highlight it. For example, the hematogones and the leukemia cells have a significant degree of immunophenotypic overlap, which is the problem. So, for example, the hematogones are not a distinct population. They are maturing. So, as they mature, the hematogones tend to have decreasing intensity of CD9, CD10, CD19, the CD38. The CD45, is increasing. CD34 is decreasing. CD20 is acquiring.

Now the way we are able to differentiate the leukemia population is for that degree of maturation, what is the aberrancy? So, it could be overexpression of CD19 or underexpression of CD38, for example. Or it could be abnormal coexpression of two different markers. For example, CD20 on the CD34-positive cells. You're not expected to see that. Or it could be that these cells are expressing aberrant myeloid markers like CD13, CD33, etc.

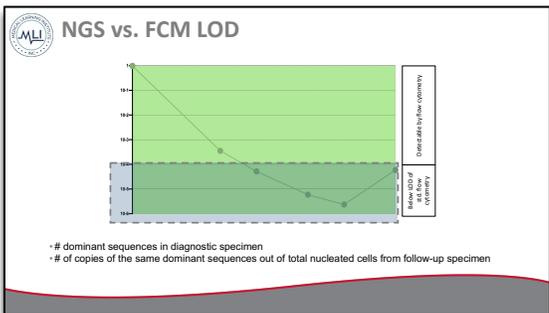
I wanted to show in the plots that the gray cells are the hematogones, regenerating in this 28-year-old man. And it's just the red cells that are CD34 bright, CD10 bright, CD38 decreased, and CD66 C-positive that are actually the positive MRD.

**Sample Flow Cytometry Report**

Accession Number:  
 Collection Date and Time:  
 Received In-lab Date:  
 Specimen Type:  
 Interpretation: POSITIVE FOR MEASURABLE RESIDUAL B-ALL  
 Comments:  
 Nucleated Events Acquired: 907109  
 Aberrant cells are 0.025% of total analyzed events.  
 Aberrant Population: Blasts  
 Aberrant Cell Phenotype: .....

### Sample Flow Cytometry Report

Now the flow cytometry from the pathologist perspective should be comprehensive enough to provide an informed report. In addition to the patient details and interpretation, it's important to note the nucleated events acquired so that the oncologist is aware that the sample was adequate and the degree of MRD.



### NGS vs FCM LOD

This is just a plot comparing the sensitivities of NGS versus flow cytometry. As this is not new for the audience here, the positive values in the lower box is below the detection limit of flow cytometry, NGS is able to identify them.

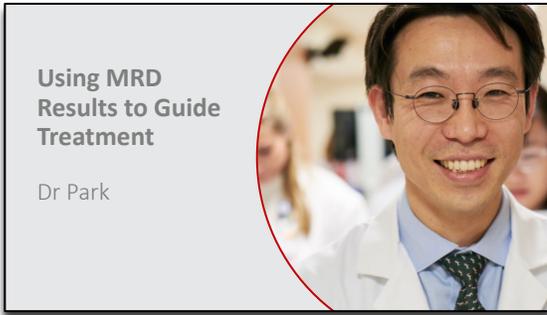
**Assessment**

A patient with ALL receives an MRD result of 0.025% leukemic cells. Is there support for MRD?

- A. Yes, positive for MRD
- B. No, this is below the MRD threshold
- C. Unknown, the method used for analysis was not defined
- D. The data provided are irrelevant because the results do not change management

### Assessment

A patient with ALL receives an MRD result of 0.025% leukemic cells. Is there support for MRD? This is positive for MRD. Flow cytometry sensitivity is about 0.01. It's hard to go below that.



### Risk Stratification of Patients

- MRD is often a better indicator of the risk of relapse than standard baseline characteristics, such as white blood cell count, immunophenotype, or cytogenetics<sup>1</sup>
  - Caveat: some genetic aberrations greatly affect risk of relapse even in MRD-negative patients<sup>1</sup>
- Treatment regimen can be altered according to post-induction MRD status and the time to MRD negativity<sup>1-4</sup>
  - To increase the cure rate among patients with a high risk of relapse, can consider
    - Intensification of therapy with HSCT in CR1<sup>2</sup>
    - Intensification of chemotherapy<sup>2</sup>
    - Introduction novel therapies, such as blinatumomab or inotuzumab ozogamicin<sup>2,3</sup>
  - In contrast, patients who rapidly achieve MRD-negativity may be good candidates for less intensive treatment
    - Avoidance of HSCT and its related toxicities and increased mortality

### Risk Stratification of Patients

Dr. Park: So, we'll finish the presentation today about what to do when you get the MRD-positive results. I know I've got some questions about the treatment decisions. So, we talked about why it is important to test already, the dynamic nature of it.

### Use of High-Throughput Sequencing Improves Risk Stratification<sup>1</sup>

- In pediatric patients with B-ALL (n=619), HTS identified a subset (n=55) who were FCM-MRD-/HTS-MRD+ at ED1<sup>1</sup>
  - These patients had inferior 5-year EFS
  - Considered as higher-risk and possible candidates for intensification of therapy
- Another subset of patients (n=56) had undetectable MRD at a 0.001% cutoff
  - 5-year OS = 100%
  - May be candidates for treatment reductions instead
  - Importantly, the HTS-MRD- patients in the HR population did NOT show the uniformly 100% OS

In this study, HTS identified higher-risk and very low-risk patients

### Use of High-Throughput Sequencing Improves Risk Stratification

I think one of the big questions is did the role of NGS versus multiparameter flow cytometry, which is 10<sup>-4</sup>? Now we have the tools to get even deeper.

So, what do you do for those patients who are flow-negative with 10<sup>-4</sup> but who are positive, now detectable disease

essentially below the level of 10<sup>-4</sup>? So, there are more emerging data. That level of sensitivity helps us further distinguish patients who are even at higher risk. The flow-negative but NGS-positive patients are somewhere in between patients who were NGS-negative and then flow-positive.

That's one patient population we may want to think about intensified therapy. None previously were called MRD-negative. But those patients who were truly NGS-negative and the even deeper level that we can now think about maybe deintensifying the therapy because these patients actually do very well.

So, this is the data from the pediatric ALL. Now we need more data, prospective data, such trials are ongoing.

### Blinatumomab in MRD+ B-ALL<sup>1</sup>

**Eligibility criteria**

- 1st or later CR AND
- Persistent or recurrent MRD  $\geq 10^{-3}$  after minimum 3 blocks of intense chemo

**Primary endpoint**

- MRD-CR after 1 cycle

**Secondary endpoint**

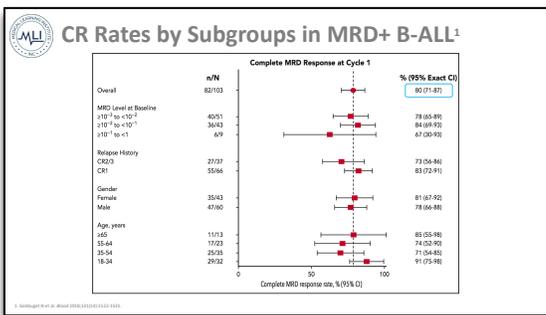
- RFS at 18 months

Characteristic	Patients (n=116)
<b>Relapse history, n (%)</b>	
In first CR	75 (65)
In second CR	39 (34)
In third CR	2 (2)
<b>Baseline MRD levels</b>	
$\geq 10^{-1}$ to $< 10^{-2}$	9 (8)
$\geq 10^{-2}$ to $< 10^{-3}$	45 (39)
$\geq 10^{-3}$ to $< 10^{-4}$	52 (45)
$< 10^{-4}$	3 (3)

### Blinatumomab in MRD+ B-ALL

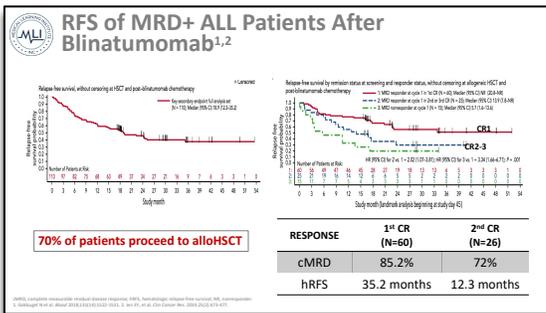
So, in terms of the therapy, blinatumomab is the only approved one for now. This was the pivotal clinical trial; it was international study. The patients eligible for the study were first or later CR, although the vast majority of the patients were in first CR, about 65%. But there are patients who are entering the study at the second or the third relapse with MRD-positive. In order to enter this study, the cutoff was  $10^{-4}$ , so slightly above our typical MRD-positive, MRD-negativity; to truly be confident they were actually getting MRD-negative you have to start a little bit higher at a detectable level.

they were actually getting MRD-negative you have to start a little bit higher at a detectable level.



### CR Rates by Subgroups in MRD+ B-ALL

The response rate was 80%, so blinatumomab was effective in converting MRD-positive to negative in 80% of the patients, across all MRD levels and relapse histories and gender, and regardless of the age as well.



### RFS of MRD+ ALL Patients After Blinatumomab

So, this is the data for the relapse-free survival for those patients. The majority of the patients did go to allogeneic transplant, so we do consider blinatumomab as a bridge to consolidative allogeneic transplant for vast majority of the patients. However, it is encouraging, even with the longer outcome, that we are curing more of the patients, about 50% of the patients, as opposed to traditional MRD-positive patients which we would have expected about 20% survival for those.

The other take-home point for me was that the patients in the CR1 did better than CR2 and CR3, which may be intuitive that the patients do better in the earlier remission status. But this is also pointing at the role of MRD assessment. Switching therapy is even more critical in the first line of a therapy than second and third line. Although you can still make a difference in the patients' lives. The biggest difference in the cure rate we can make is in the first CR or the first-line therapy. So, it's even more important that we are vigilant monitoring the MRD and making appropriate therapy change.

### FDA Approval of Blinatumomab for MRD+ B-ALL in US

- Blinatumomab approved for the treatment of B-ALL in first or second complete remission with MRD  $> 0.1\%$ <sup>1,2</sup>
- Prior to the approval, MRD results did not change patient management<sup>3</sup>
- With this approval, MRD incorporated as standard of care for all subtypes of ALL
- In January 2020, the FDA released guidance for industry on the use of MRD in the development of investigational agents for hematologic malignancies<sup>4</sup>
  - FDA accepts MRD levels of  $< 0.01\%$  as evidence of efficacy
  - ALL is the only disease in which MRD has been used as a surrogate endpoint supporting drug approval

### FDA Approval of Blinatumomab for MRD+ B-ALL in US

So, as I mentioned before, the approval for blinatumomab is in this indication. The FDA has released guidance for industry on the use of MRD. MRD is being used as an endpoint, and CD19-targeted CAR T-cell therapy is being studied for the indication of an MRD. The FDA has accepted the MRD level of less than 0.01% as evidence of efficacy in ALL.

### Inotuzumab Ozogamicin in Adults With MRD+ B-Cell Precursor ALL – Study Design and Patient Characteristics<sup>1</sup>

- Adults with B-cell precursor ALL in MRD+ CR (N=16)**
  - CR1 (n=11)
    - Did not achieve MRD negativity or who experienced MRD recurrence after 23 mo from start of frontline therapy
  - CR2+ (n=5)
    - Experienced MRD relapse after 1 month from start of salvage therapy
- Inotuzumab ozogamicin**
  - 0.6 mg/m<sup>2</sup> D1 and 0.3 mg/m<sup>2</sup> D8 in cycle 1
  - 0.3 mg/m<sup>2</sup> on D1 and D8 in subsequent cycles up to 6<sup>o</sup>
- Endpoints**
  - MRD negativity
  - OS
  - RFS

Patient characteristics, n (%)	Inotuzumab ozogamicin N=16
Ph+ ALL	10 (63)
Received concomitant TKI	
Ponatinib	9
Dasatinib	1
Persistent MRD	10 (62.5)
MRD recurrence	6 (37.5)
Prior therapy	
Blinatumomab	9 (56)
AlloHsCT	3 (19)
CAR T-cell therapy	1 (6)

## Inotuzumab Ozogamicin in Adults with MRD+ B-Cell Precursor ALL – Study Design and Patient Characteristics

So what agents, other than blinatumomab, are being evaluated? I already talked about CD19-specific CAR T-cells. There are some data that CAR T-cells lower the disease burden and MRD level even greater with less toxicity.

Inotuzumab has also data in MRD-positive B-cell ALL. This is data from MD Anderson by Nick Short. It was presented at ASH last year. It's a smaller study of 16 patients at a lower dose of inotuzumab compared to our full on morphologic relapse dosing. But when the inotuzumab was used at a lower dose, it was still effective in the majority of the patients.

The patients were 63% Ph+, and because of that, they had a TKI before. About 56% of the patients had a prior blinatumomab and then had persistent MRD or recurrent MRD for which they received inotuzumab. And about 1 patient or 6% of the patients had a CD19 CAR T-cell therapy.

### Inotuzumab Ozogamicin in Adults With MRD+ B-Cell Precursor ALL – Efficacy<sup>1</sup>

Inotuzumab ozogamicin (N=16)	n (%)
MRD-negative at any time	8 (50)
Ph- ALL (n=6)	4 (67)
Ph+ ALL (n=10)	4 (40)
MMR as best response	4 <sup>a</sup>
Response by prior therapy	
Prior blinatumomab (n=9)	3 (33)
No prior blinatumomab (n=7)	5 (71)
Received alloHsCT	5 (31) <sup>b</sup>

## Inotuzumab Ozogamicin in Adults with MRD+ B-Cell Precursor ALL – Efficacy

In terms of the efficacy, the conversion rate was about 50%, so slightly lower than blinatumomab; but we also have to remember this is also a different patient population. The patients who had prior blinatumomab and were then either refractory or recurrent MRD afterwards, had a slightly lower response rate of 33%. What about those patients who are

MRD-positive after blinatumomab? What do you do afterwards? If they're CD19-positive, you can also consider CAR T-cells for those patients, either in a clinical trial or with a commercial therapy. Inotuzumab could be an option in CD22-positive patients based on this data as well.

### Stem Cell Transplant Based on MRD Status

- T-ALL and Ph-negative B-ALL
  - HsCT leads to greater relative improvement in outcomes in patients who are MRD-positive in CR1 compared to MRD-negative patients<sup>1</sup>
  - Though, patients who are MRD-negative still have superior outcomes<sup>2</sup>
  - In one study, MRD-positive patients without HsCT had the lowest probability of RFS and OS, while MRD-positive, HsCT patients had RFS and survival more similar to MRD-negative patients<sup>3</sup>
- Ph-positive B-ALL
  - HsCT does not seem to benefit patients who are MRD-positive<sup>4</sup>

## Stem Cell Transplant Based on MRD Status

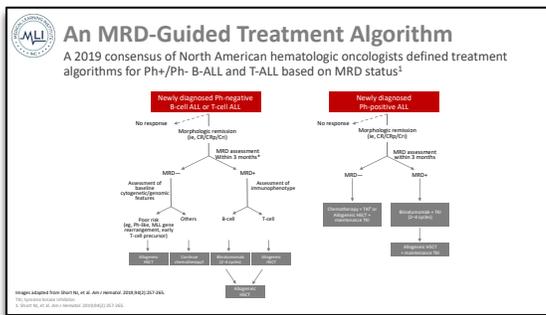
Once you're MRD-positive, you make the switch to blin or inotuzumab, and the vast majority of the patients will probably end up receiving blinatumomab. And then if they do achieve MRD-negativity, the goal is for these patients to go to allogeneic transplant afterwards. So, the data show that patients who are MRD-negative are able to do better. So, if you're actually able to convert the MRD-positive to negativity and then go to

transplant, the outcome of those patients is much better than for patients who had a persistent MRD at the time of a transplant.

The tricky question for all of us is, are we confident that we have the tools to convert the MRD-positive to MRD-negative? For blin-naïve patients, the answer is probably straightforward. But patients who had previously received blinatumomab don't have a lot of options. We may just have to go to transplant

because there's no other effective therapy that we're confident about converting to MRD-positive to MRD-negative.

There's also data, this is kind of buried in here a little bit, MRD-negative patients actually didn't benefit much from the transplant. The biggest benefit of the transplant is for MRD-positive patients. For those high-risk patients, we're able to convert them into the risk profiles. But, again, this speaks to the point that with truly good or extremely good responders, we may be able to deintensify the therapy and not send these patients to transplant. And that may end up being true for some of the Ph-positive B-cell ALL, which used to be considered very high-risk disease. But given the blinatumomab and the chemotherapy approach, the vast majority of these patients are actually getting molecular remission. So, we are actually now sending these patients automatically to consolidative allogeneic transplant in CR1 if they do achieve a deep molecular CR at the level of  $10^{-6}$  early on at month 3 and month 4.

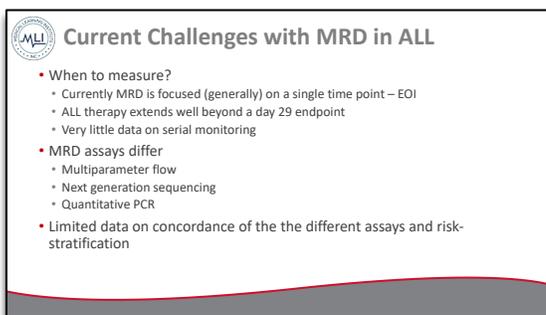


### An MRD-Guided Treatment Algorithm

So, one of the last slides is the practical guidelines for one of the publications from the 2019 consensus. It is similar to what was in the NCCN panel, and the idea is actually very simple, probably too simplified. But in the morphologic remission patients after induction or consolidation therapy, if they're MRD-negative, they usually stay with the current therapy. If you're MRD-positive, we are switching to blinatumomab, which is one approved indication for therapy.

For T-cell ALL, which we didn't talk about much the guideline is to go directly to allogeneic transplant. However, I would argue that for relapsed T-cell ALL patients, we have very few salvage treatment options. So, I really feel strongly that if we're able to convert the MRD-positive to MRD-negative, we should hold off on therapy. Meaning if they have not had nelarabine or asparaginase-based therapy, if we think we're able to do it (and this I know is a big if and sometimes not easy to do), we should try to convert them into MRD-negativity before they go to transplant because, again, if you go to transplant with MRD-positivity, the relapse rate will be higher.

For Ph-positive, as we talked about, if you are MRD-negative after a TKI-based regimen, those patients are able to stay on their current regimen or allo is an option. But as I mentioned before, early molecular remission in these patients may not need allogeneic transplants. So, we're doing a little bit less transplant for these patients, really a striking contrast to our previous approach for Ph-negative patients.



### Current Challenges with MRD in ALL

So, in summary, we talked about when to measure, which is not completely standardized because the ALL therapy in adults are not standardized, and that's one of the biggest challenges. So, when in doubt, it is really better to consult with an ALL expert at a specialized center to choose the right first-line therapy. Again, the best chance of a cure is the first time. So initial

therapy really does matter, and MRD monitoring is even more important for first-line therapy.

MRD assays do differ. We heard a lot about the differences, so it is important to think about them. As the NGS data is getting more mature, we may not need the bone marrow biopsies as much, and we may move to use peripheral blood, I think the next frontier in MRD assessment.

**Assessment**

At EO1, a 23-year-old patient with Ph-negative B-ALL is in complete remission but has an MRD of 0.1% ( $10^{-3}$ ). Which of the following treatments is most appropriate for this patient?

- Inotuzumab ozogamicin
- CD19-directed CAR T-cell therapy
- Ponatinib
- Blinatumomab
- Continued multiagent chemotherapy

### Assessment

All right, tour de force assessment at the end. So, at the end of induction, a 23-year-old patient with a Ph-negative B-ALL is in complete remission but has an MRD-positive at 0.1%. Which of the following treatments is most appropriate for this patient? Ino, CAR T, ponatinib, blin, or continue the multiagent chemotherapy? Most of you chose blinatumomab, which is correct. Continuation of the therapy would have been incorrect if they're MRD-positive at that point.

**Assessment**

In which of the following ways is MRD status useful for guiding treatment management?

- Treatment reduction
- Incorporation of a tyrosine kinase inhibitor in treatment
- Incorporation of blinatumomab in treatment
- All of the above
- Only A and C

### Assessment

Which of the following is MRD status useful for guiding treatment management? One, treatment reduction. Incorporation of TKI in treatment. Incorporation of blin treatment, all of the above, or only A or C. Most of you chose only A and C. I think the TKI could also be a reasonable option. I don't think that's necessarily wrong. I think we have a little bit less data for actually switching to TKI for the MRD indications. So, on the relapsed cases, morphologic relapse, we will often do that.

**Assessment**

Because patients who are MRD-positive have a greater chance of relapse after hemopoietic stem cell transplant than MRD-negative patients, this therapy is not recommended for MRD-positive patients.

- True
- False

### Assessment

Because patients who are MRD-positive have a greater chance of relapse after hematopoietic stem cell transplantation than MRD-negative patients, this therapy is not recommended for MRD-positive patients. True or false?

**Key Takeaways**

- In ALL, 30% to 50% of patients have residual leukemic cells that contribute to eventual relapse
  - Pediatric and adult patients who are MRD-positive consistently experience worse outcomes than those who are MRD-negative
- MRD should be measured at multiple times in ALL, including at diagnosis, after induction, after consolidation, before transplantation, and at regular intervals during maintenance and surveillance
- Multiple techniques are available to evaluate MRD, each with advantages and limitations
- Awareness of these limitations is important when choosing the correct method to enable precise MRD measurement
- For patients who are MRD-positive, the goal is to eradicate MRD with MRD-specific therapy—specifically blinatumomab—before stem cell transplant

### Key Takeaways

In summary, we emphasized MRD assessment and its prognostic importance, some of the assays that we do, and their limitations. If you're using NGS, we do need to send the diagnostic samples to analyze them better. You have to think about these assays when you first meet the patient or do the original diagnostic bone marrow.



## Q&A

**Speaker:** Specifically for early precursor T-cell ALL, do you still recommend, according to the algorithm, transplant even if they're MRD-negative after four weeks?

01:09:49

**Dr. Park:** Yes, thank you for that question. So, we talked a lot about MRD-negative, but there is one caveat which I didn't talk about. There are very high-risk patients, who despite MRD-negativity we worry about. I agree with your early T-cell

precursor that T-ALL is one of them. And for B-cell ALL, we are learning more about them. Traditionally, 4:11 translocation or MLL gene arrangement and a hypodiploid ALL, based on the pediatric data, are the two common indications that we automatically send to transplant, even if they're MRD-negative.

I don't know whether blin and ino in front-line therapy still exists; they're still worse prognosis because risk was based on multi-agent chemotherapy. So that may very well change, but currently given the lack of data, those are the two groups that I'm sending to transplant.

If early MRD-negative, do they really need to go to transplant? Ph-like or p53 mutation are some of the other indications for transplant, despite MRD-negativity. So, there is a caveat for very high-risk groups proceeding to transplant.

**Speaker:** Even if they have been treated with pediatric-inspired AYA regimens? You still send?

**Dr. Park:** That's also a good question. If an MRD-negative younger patient is on their regimen, if they're following the published clinical trials, I do not. But if those patients are receiving hyper-CVAD, then I would do. I think the long-term data is not as good for that group. But for the pediatric intensified therapy, I would follow the clinical trial.

**Speaker:** Thank you so much.

**Dr. Park:** There's one question about do you always change therapy based on MRD after induction? Wouldn't it be reasonable to wait until post-consolidation? So that is correct. Even though we assess for MRD-positivity after induction, I usually use the timepoint in adult ALL that we're not switching therapy for even the pediatric or pediatric-inspired chemotherapy.

It does give us some prognostic information as to how the patients will do, but MRD-positivity at the end of four weeks usually is not enough to switch therapy. So, you're right that this is post-consolidation, which is usually about three-month mark. This is the time that we make the switch.

But they are MRD-positive convergent in 4 to 8 weeks, 4 to 12 weeks. And those are the tricky patients we don't know quite what to do. When we looked into our data, it's a small number. It's hard to say. But as long as they convert to MRD-negativity at month 3, they may still be a good prognosis patient. But sometimes the MRD-negativity at week 4 can identify a good risk patient. So, there may be another value. In terms of switching therapy though, I agree they have to have consolidation or three-month mark will be the better one to do.



The second question was how would you treat adult patients with Ph-negative B-ALL with a persistent MRD-positivity after blinatumomab? Would you consider ino or allo transplant with positive MRD? Very hard question.

Not all MRD is the same. The lower the better, so 0.1% is different from 0.01% or at the kind of level of a detection too.

If somebody had a very high level of disease or the more than 1%, and then after blinatumomab they're nearing the MRD-negativity or they're clearing coming down, I may argue to go to transplant. But if somebody's having a very flat line or 0% or 0.1% or higher, I then will think about switching therapy to something else, even trying inotuzumab, even though there's a VOD risk. I agree this is a very difficult clinical situation, probably case by case. But inotuzumab will be probably another reasonable option.

Using miniCVD allows a little bit lower dose of inotuzumab, and then a lower risk of a VOD. Recent data about VOD risk with inotuzumab appears to be less than what was published with a change in the conditioning regimen. Avoid double alkylating therapy for transplant. So, I'm less worried about those with the reduced intensity of inotuzumab, it may be a good option.

**Speaker:** So, I, I've got a question about the definition of MRD-positivity because I thought that you showed data that if you are low level positive, that there is not really a benefit for a transplant then.

**Dr. Park:** The MRD cutoff there was 0.01%. So, if they are still greater than 0.01%, which is the current positivity, I think that the data is for transplant. But if they're less than 0.01%, and now we're picking up 0.001%, the data is less clear about the transplant.

**Speaker:** I was wondering about the patient who was still MRD-positive while on blin, whether their CD19 was still positive, or would we be looking at the CD22 levels? And also, maybe using a Capizzi-style intensification with PEG-asparaginase, increasing doses of methotrexate to assist with eradication of disease.

**Dr. Park:** ALL therapy is not one size fits all, so you're absolutely right there. I think switching the therapy really does depend on what they received previously. Because if they already received several cycles of asparaginase, it probably doesn't make sense. But if they have not, then perhaps. Those two questions do come up. With ino, which is effective, should we should we try it? But the liver toxicity with ino is one big concern.

So, I think that could be an option. But I think assessing the antigen level is really key, especially after blinatumomab or inotuzumab, to the 19- and 22-positivity because it will really impact their future treatment, including CAR T-cell therapy.

If you're 19-negative after blinatumomab, we can see the regain of those 19-positivity. Sometimes it can be falsely negative due to antibody blocking. So, it is important to monitor them later on to see the more 19-positive populations. But both for MRD assessment and for therapeutic selection, measuring antigens for 19 and 20 really is key in this day and age for ALL.

Thank you again for joining us today. I hope it was informative and you learned something good.