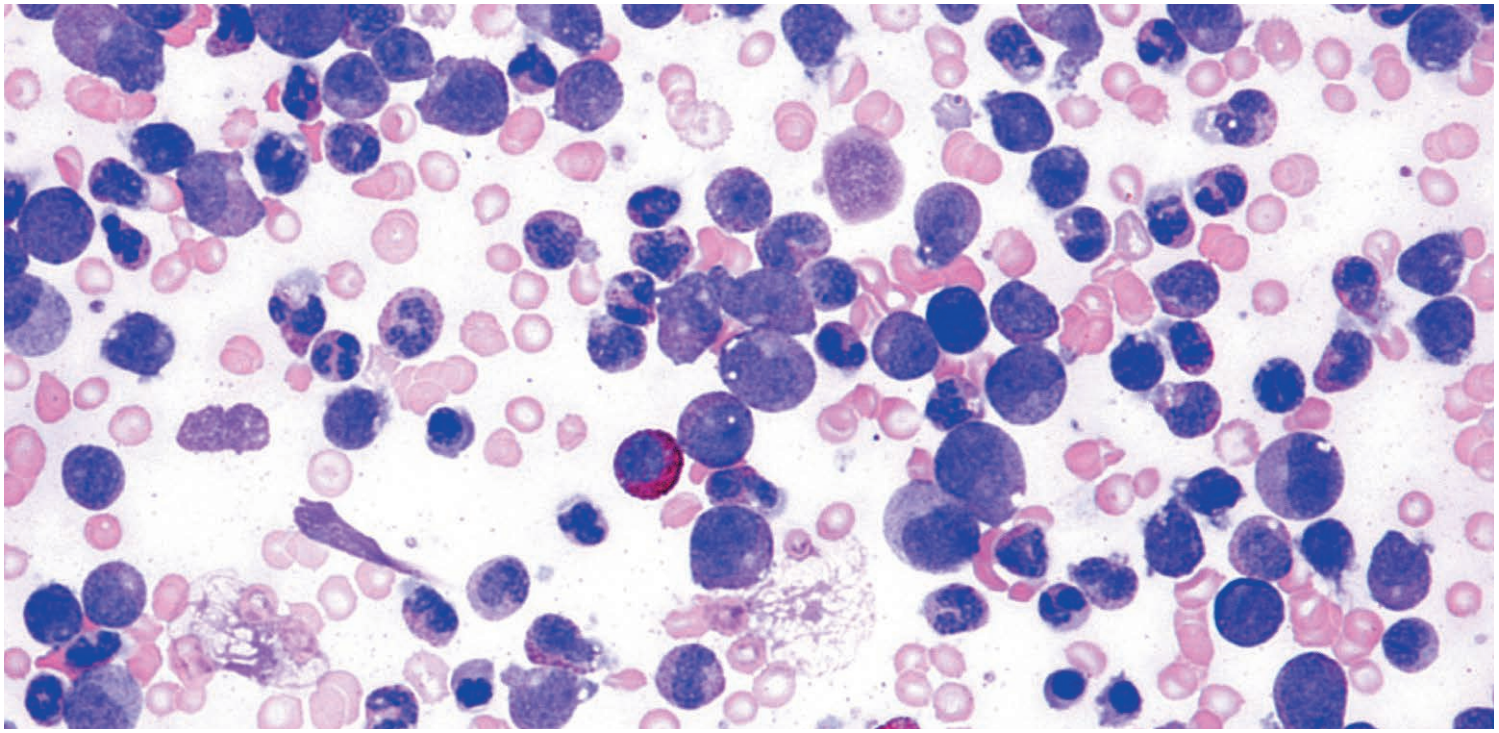

Some chronic myeloid leukemia patients can safely stop TKI therapy:

Ultrasensitive DNA detection can help determine who they are

By **Dr. Ehab Atallah, MD and Carolyn Reifsnyder**

Abstract and Background

Chronic myeloid leukemia (CML) is a form of blood cancer that affects myeloid cells within the bone marrow. As of 2017, just over 58,000 Americans were living with CML,[1] though that number is predicted to rise to 180,000 by 2050.[2] Current guidelines recommend patients undergo lifetime treatment with tyrosine kinase inhibitors (TKIs) to keep the disease in remission by inhibiting BCR-ABL1 tyrosine kinase and subsequent CML cell proliferation (details below). While effective, this means living with significant side effects long term.

The LAST study[3] (Life After Stopping Tyrosine Kinase Inhibitors) was a prospective, single-arm, non-randomized clinical trial designed to determine whether certain CML patients could safely discontinue TKI treatment without experiencing a recurrence of the disease. The study focused on 172 participants who showed a sustained deep molecular response to TKI therapy based on their levels of the BCR-ABL1 gene fusion. These individuals had a mutational burden below 0.01 percent (MR4), indicating the disease was in remission.

BCR-ABL1 gene fusion tests can run on a variety of diagnostic platforms, including RQ-PCR and Droplet Digital PCR* (ddPCR), but their relative sensitivity matters. In a comparison performed in the LAST study, results from both methods indicated that levels of BCR-ABL1 were below the detectable limit in 87 participants. Just 10.3 percent of those individuals had a molecular recurrence of CML after stopping TKIs. For an additional 56 patients, ddPCR detected levels of BCR-ABL1 that were beneath the limits of detection for real-time quantitative PCR (RQ-PCR). Of those patients, more than half (64.3 percent) developed a molecular recurrence and had to resume TKI therapy.

Introduction

Chronic myeloid leukemia (CML) is a form of cancer that affects myeloid cells within the bone marrow that are responsible for forming blood cells. According to the National Institutes of Health, there were an estimated 8,450 new CML cases diagnosed in the U.S. in 2020. Overall, about 1 in every 500 people will be diagnosed with CML in their lifetimes.[1] CML primarily affects adults, with around half of new patients being 65 or older.[5]

Understanding CML as a disease and how it can be treated has a rich and lengthy history that has broadly influenced the field of oncology. It was through the study of CML and the discovery of the Philadelphia chromosome in 1960 that scientists first learned of the link between chromosomal abnormalities and the development of cancer.[6] Further research revealed that the aberrant chromosome was in fact a translocation between chromosomes nine and 22, which visibly changed the chromosomal architecture when examined under a microscope.[7] When evaluated by molecular means, the Philadelphia chromosome forms an abnormal gene: BCR-ABL1. The aberrant gene product produces a hyperactive tyrosine kinase that affects the formation of new blood, including red blood cells, platelets, and many types of white blood cells.

Because CML symptoms all stem from a hyperactive tyrosine kinase, tyrosine kinase inhibitors (TKI) emerged as an effective way to manage the disease. As the name suggests, this class of drugs directly inhibits tyrosine activity. The first, imatinib, became available about 25 years ago.[8] Imatinib and its successors (e.g., dasatinib, nilotinib, and bosutinib) have dramatically changed the outlook for this disease. Before TKIs, patients diagnosed with CML had a life expectancy of just six years. Now, with TKI therapy, patients' life expectancy is similar to the general population.[9] While this dramatic increase in survival rate represents a remarkable success, it also means that a growing number of people are receiving TKI therapy on an ongoing basis. For routine care, these individuals are monitored over time to quantify their disease burden and ensure that the treatment is working.

Diagnosis and monitoring for CML consists of a relatively straight-forward molecular test for the BCR-ABL1 gene fusion. These tests today can run on a variety of diagnostic platforms – including PCR, RT-PCR and Droplet Digital PCR (ddPCR) – with an aim to detect the gene in patient blood to measure mutational burden. Low levels of BCR-ABL1 indicate reduction in severity of the disease: A patient with undetectable levels may be in remission, while higher levels of the gene indicate active disease. A subset of patients will show a sustained deep molecular (DMR) response to TKI therapy, where repeated tests in a row show that their mutational burden is below 0.01 percent BCR-ABL1, i.e., a greater than four-log reduction according to the international scale (**Figure 1**). Patients that display DMR over time may remain in this state indefinitely.

Glossary

Term	Definition
Major molecular response	BCR-ABL1 levels at or below 0.1 percent according to the International Scale
MR4	BCR-ABL1 levels < 0.01 percent, i.e., greater than four-log reduction in BCR-ABL1 levels
Deep molecular response (DMR)	Repeated test results demonstrating MR4
Sustained DMR	Ongoing MR4 for at least two years
Molecular recurrence	Loss of major molecular response (BCR-ABL1 International Scale ratio > 0.1 percent) by central laboratory testing
Treatment-free remission	Major molecular response in the absence of TKI treatment
Patient reported outcomes (PROs)	Self-reported select experiences and health parameters

Figure 1: Terms used to describe the molecular and experimental states of CML Patients.

Some early studies have reported that a subset of patients exhibiting a DMR can successfully stop TKI therapy and remain in remission.[10-15] Until recently, however, there was insufficient evidence for clinicians for the reliable identification of this subset of patients. To err on the side of safety, almost all CML patients were directed to stay on TKI therapy for the rest of their lives.

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The Next Evolution of CML Treatment

Reducing CML symptoms when the disease firsts manifests is still paramount. TKI treatment is currently the most effective option, but it's important to recognize that patients experience a range of side effects that can diminish their quality of life. This a huge burden to carry, particularly given its current use as a life-long medication.

While TKIs are touted as drugs that allow patients to live a normal life, patients themselves often report that the drugs have reduced their quality of life. Some effects may be considered subtle; however, some effects are significant, especially to patients who need to stay on drug indefinitely. TKI treatment affects patients' lives in countless other ways, both large and small – from the minor inconvenience of having to remember to take a pill every day, to the more life-altering. For example, patients may have lower exercise tolerance, so they are unable to play sports or run; instead of enjoying a long drive, the constant threat of diarrhea makes traveling stressful. Instead of feeling stable, some feel emotionally drained and chronically depressed. Individuals taking TKIs are instructed to not get pregnant as the drug increases the risk of miscarriage. Furthermore, TKI treatment is expensive, costing up to $100,000 per year per patient. Few can easily afford this expense, rendering most patients utterly dependent on their insurance and fearful of changing jobs in case the switch causes a break in their coverage. Overall, there is a lot to gain if TKI treatment can be safely stopped once a sustained and efficacious deep molecular response is achieved.

As more is learned about CML, the next frontier for those studying the disease is to find instances when TKI treatment can safely be stopped or reduced to improve patients' *quality of life*. In support of this, TKI discontinuation trials, like the LAST study, seek to help patients and their clinicians determine (a) if a patient is in sustained DMR and therefore qualifies to consider TKI discontinuation and (b) whether or not they feel comfortable stopping what would have

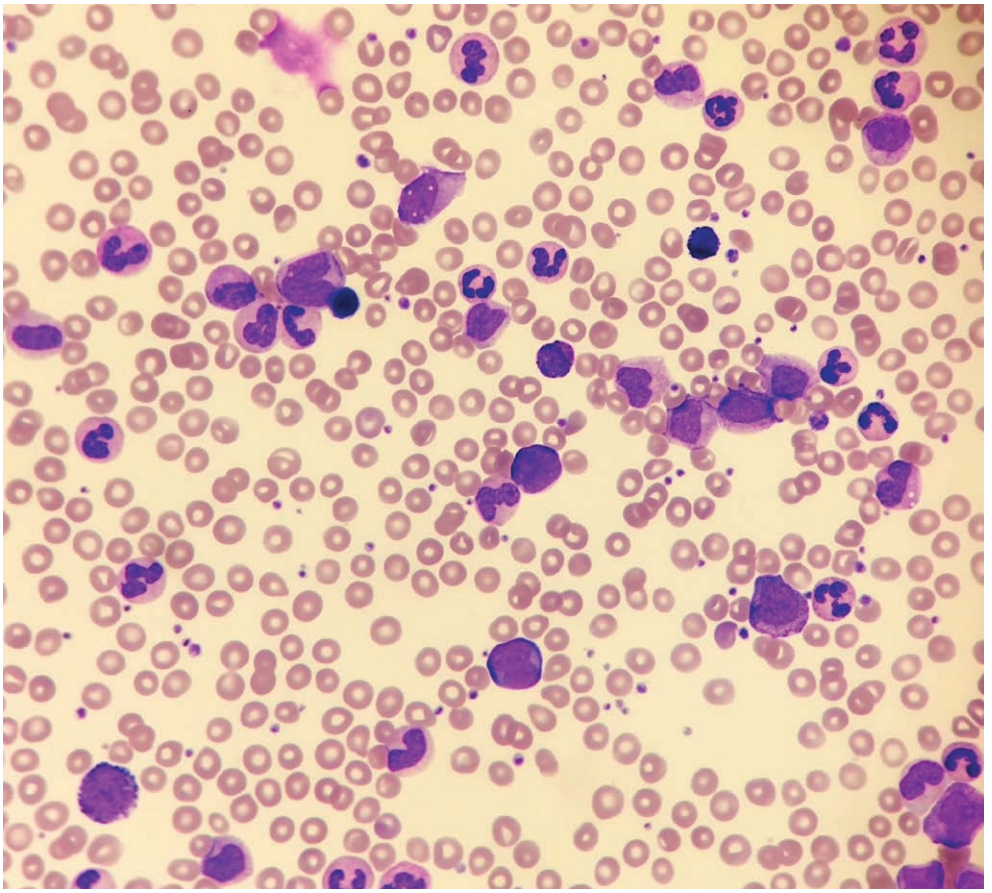
been a lifetime therapy, replacing it with ongoing monitoring for the BRC-ABL1 biomarker instead.

The LAST Study

The LAST study was designed to both gather patient reported outcomes (PROs) that describe how TKI discontinuation changed a patient's health status and to determine what proportion of U.S. patients with CML achieve treatment free remission (TFR).

It was also the largest, and only, prospective U.S.-only TKI discontinuation study to date. Some 173 adult, chronic-phase CML patients were enrolled in the study between 2014 and 2016, with 172 participating in follow-up activities for three years or more. Participants were between 21 to 86 years of age, with a median age of 60. About half of the patients identified as women. The study had broad enrollment requirements in that it was open to individuals on a range of different kinds of TKIs, rather than just one. Specifically, all patients were taking one of four common first or second generation TKIs – imatinib, dasatinib, nilotinib, and bosutinib – for three or more years. No patients had developed TKI resistance, and their disease was considered well controlled, with at least two years of well-documented PCR tests indicating BCR-ABL1 levels at less than 0.01 percent.

The study involved 14 different medical institutions but used a centralized lab to evaluate samples. The LAST study demonstrated that, not only was this strategy possible, but the use of a single lab to run all samples provided more consistent, accurate results. At the start of the study, all patients were evaluated for BCR-ABL1 with real-time quantitative PCR (RQ-PCR) using two separate tests run at least three weeks apart.



Molecular Analysis

Enrolled patients discontinued their TKI treatment, and their blood samples were monitored closely throughout the course of the study for signs of molecular recurrence, defined as BCR-ABL1 levels rising above 0.1 percent, or the loss of major molecular response. RQ-PCR was used as the primary mode of testing to track their disease state and allow for a rapid response should molecular recurrence occur. Initially, BCR-ABL1 levels were tested on a monthly basis, but over the course of three years, the interval lengthened to every three months. During the study, some patients displayed molecular recurrence and had to restart long-term TKI treatment. Those participants were then tested once every three months by RQ-PCR until their BRC-ABL1 levels dropped back down to 0.01 percent for two tests in a row, as they had been prior to the start of the study.

After discontinuing TKI treatment, 112 of the 172 participants (approx. 65 percent) stayed in major molecular response for at least three years; 104 of those remained in treatment-free remission. Eight patients who were still in major molecular response opted to restart TKI therapy for reasons such as withdrawal syndrome, as well as physician or patient anxiety that the disease would resurface. Conversely, 60 patients (approx. 35%) displayed molecular recurrence. All but one of those participants restarted TKI therapy (**Figure 2**). Of the patients who experienced molecular recurrence, the vast

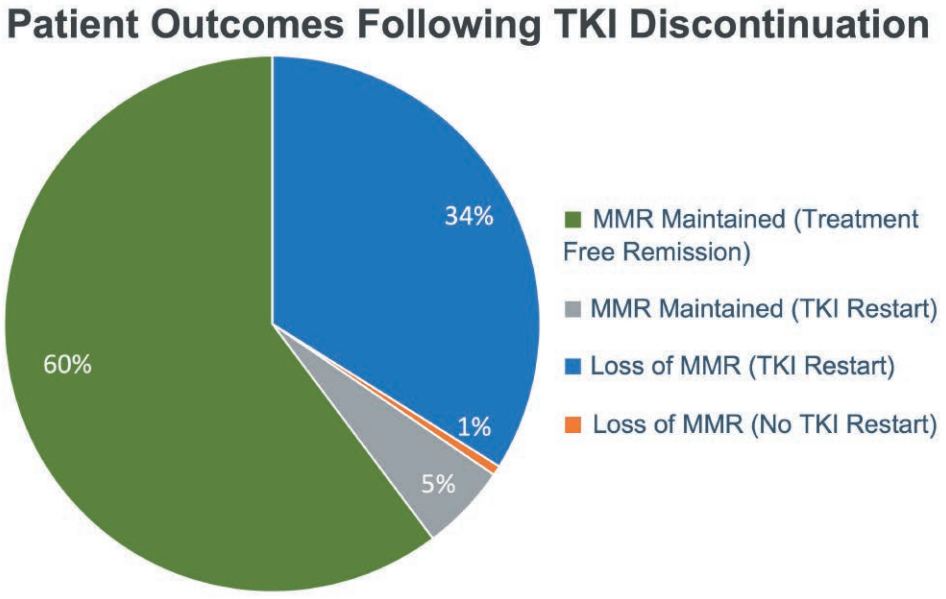


Figure 2: The spread of molecular outcomes and treatment paths for patients throughout the course of the LAST study. MMR = Major molecular response.

majority, 81.4 percent saw it happen within a year of TKI discontinuation.

While RQ-PCR is a robust testing method, there are differing opinions as to whether low levels of BCR-ABL1 measured via RQ-PCR can accurately predict molecular recurrence of CML. Without consensus, this testing approach hasn't been put into clinical practice. To address this issue, the LAST study used a method with a lower limit of detection, Droplet Digital PCR (ddPCR), to further analyze samples that tested negative for BCR-ABL1 via RQ-PCR.

A large part of ddPCR's sensitivity comes from a key step in which an aqueous sample is first emulsified into oil. This partitions the sample's nucleic acids into about 20,000 nanoliter-sized droplets; each droplet contains about one nucleic acid molecule during the reaction. The sample is then amplified using normal PCR methods. Droplets that are positive for a target gene, in this case, BCR-ABL1, light up (fluoresce) and can be counted, one by one, in a droplet reader. At the end of the reaction, the data are analyzed by Poisson statistics to yield a quantitative measurement of the number of target nucleic acid molecules in the original sample. By our estimation, this method offers about 0.5 to 1 log greater sensitivity when detecting a target gene compared to RQ-PCR.[3]

To maximize the utility of the added sensitivity of ddPCR, the LAST study set out to determine if this technology could be used to help predict a patient's risk of molecular recurrence based on an accurate analysis of their mutational load. This was accomplished by assessing patient samples using RQ-PCR and ddPCR at the start of the study and tracking how these initial results corresponded with molecular recurrence over the course of the trial.

In general, molecular recurrence was highly associated with the clinical ability to detect the BCR-ABL1 gene at the start of the study, using either tool. Of the 28 participants who had detectable levels of BCR-ABL1 at the start via either method, 50 percent experienced molecular recurrence. Of the 56 patients whose had no detectable BCR-ABL1 according to RQ-PCR, but detectable transcript by ddPCR, 64.3 percent developed molecular recurrence. Remarkably, of the 87 patients for whom BCR-ABL1 was undetectable via RQ-PCR and ddPCR, only 10.3 percent of these patients went on to develop molecular recurrence (**Figure 3**). Together, these data suggest that ddPCR results may enable clinicians to identify patients who are at particularly low risk of molecular recurrence in the absence of TKI therapy. Those individuals are likely to remain in major molecular response without treatment, potentially sparing them years of TKI side effects.

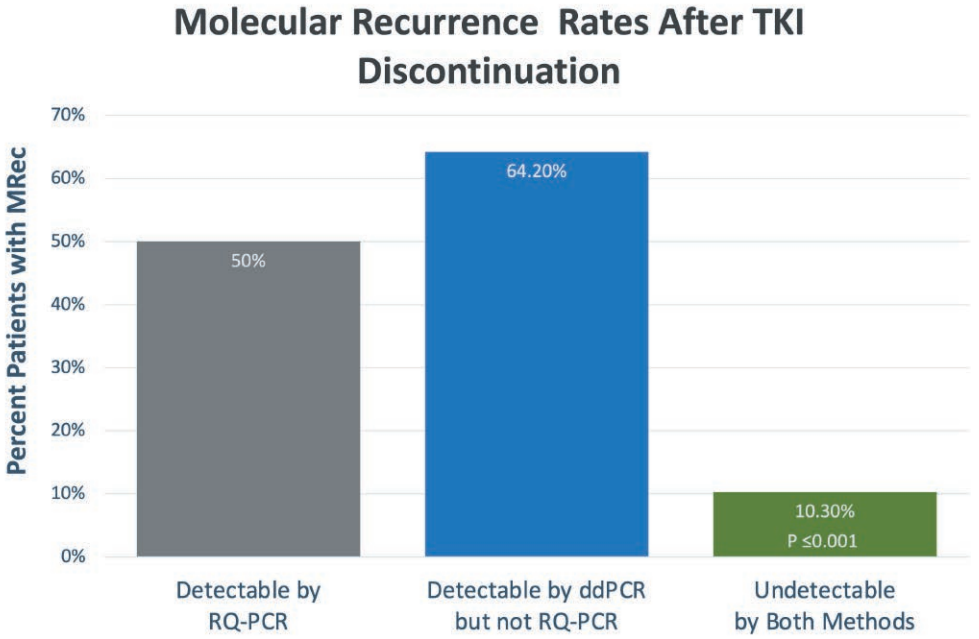


Figure 3: Molecular detection of BCR-ABL1 to TKI discontinuation is associated with molecular recurrence.

"Remarkably, of the 87 patients for whom BCR-ABL1 was undetectable via RQ-PCR and ddPCR, only 10.3 percent of these patients went on to develop molecular recurrence. Together, these data suggest that ddPCR results may enable clinicians to identify patients who are at particularly low risk of molecular recurrence in the absence of TKI therapy. Those individuals are likely to remain in major molecular response without treatment, potentially sparing them years of TKI side effects."

Patient Reported Outcomes

The LAST study was the first of its kind in the U.S. to include a comprehensive measurement of patient reported outcomes (PRO) to accompany the molecular data gathered above. This study produced a record over time for how five key symptoms and side effects changed when participants stopped TKI therapy, as well as in cases where TKI therapy was restarted. By asking patients to fill out regular assessments alongside BCR-ABL1 testing, the LAST study was able to monitor changes in fatigue, depression, sleep disturbance, diarrhea, and a more recently reported syndrome – pain interference (associated with the degree to which pain affects CML patients' lives). These variables were all independently evaluated during the study. To standardize scoring of patient responses, feedback was assessed via the PROMIS (Patient-Reported Outcomes Measurement Information System) criteria. Using this scale, a score of 50 corresponds to the mean of the general U.S. population with a standard deviation of ten. Based on the sample size of the LAST study, 172 patients, a change of three points in any of these parameters was considered clinically meaningful.

After TKI discontinuation, PRO assessments showed a significant mean decrease in four well-documented TKI side effects: 80.4 percent of patients in treatment-free remission saw an improvement in their fatigue, 87.5 percent saw an improvement in diarrhea, 34.8 percent saw an improvement in depression, and 21.4 percent saw an improvement in sleep disturbance. No mean change was seen in patient-reported pain interference. The observed improvements were sustained over time, but unsurprisingly, restarting a TKI therapy resulted in mean worsening of these PROs. In particular, patients reported minimal changes in depression, but an increase in the levels of fatigue, diarrhea, and sleep disturbance they experienced that peaked within a year of restarting TKI therapy but then got better over time. Of note, they reported higher levels of pain interference upon restarting TKIs, but these improved over time as well. These results highlight well-recognized symptoms that may be alleviated in patients that qualify to stop TKI therapy. They also highlight the need for further research into

the phenomenon of pain interference to better understand how it manifests in CML patients that are on and off TKI therapy.

Conclusion

The results of the LAST study indicate discontinuation of TKI treatment is a viable option for CML patients in a deep molecular response, so long as they are monitored carefully for molecular recurrence. Furthermore, according to the PRO measurements that were conducted during the LAST study, discontinuing TKI therapies can provide a meaningful improvement in drug side effects and quality of life. By assessing the individual's disease history, their current status and symptoms, and their risk of molecular recurrence, doctors can support patients as they decide whether discontinuing TKI treatment is a desirable option and an acceptable risk.

Patients that test negative for BCR-ABL1 by ddPCR are excellent candidates for discontinuing TKI treatment because these results are associated with a much lower risk of experiencing molecular recurrence. However, patients who have been in sustained DMR according to other clinical PCR-based methods can also safely stop taking TKIs. Some of these patients will remain in treatment free remission indefinitely, but for those who do experience molecular recurrence, restarting TKI treatment is a reliable method to return them to major molecular response status.

Despite these benefits, this study identified a clear need for understanding the complex and emotional decision to discontinue an anti-cancer drug. While some patients felt relieved to stop taking TKIs, others felt anxious that it their disease might resurface. Any clinician guiding a patient through this decision-making process must be sensitive to this anxiety and withhold judgment from patients who chose to remain on TKIs, despite being in sustained major molecular response.

Our results indicate that ddPCR has an untapped potential to help alleviate these anxious concerns. Currently, this technique is being used somewhat interchangeably with other testing methods to monitor for BCR-ABL1, however, these findings suggest that patients who test negative for BCR-ABL1 by ddPCR are at a significantly lower risk of molecular recurrence. In the future, particularly as ddPCR instruments become increasingly accessible to clinical labs, successive negative ddPCR results may reduce the amount of time patients must undergo TKI therapy while in major molecular response before their doctors consider them eligible to stop taking TKIs. Ongoing negative ddPCR results may also reduce the need for frequent testing while a patient is in treatment-free remission. For this study, patients were requested to undergo testing every one to three months. With the remarkable sensitivity of ddPCR, there may be an opportunity to expand that window of time as long as a patient continues to have undetectable BCR-ABL1 levels. Further research is needed in this area.

"Despite these benefits, this study identified a clear need for understanding the complex and emotional decision to discontinue an anti-cancer drug."

Despite mounting evidence, many patients and physicians may still prefer to "err on the side of caution" when it comes to taking TKIs, even if the patient is in a state of sustained molecular response. There is valuable information for clinicians to take on board, but for evidence-backed changes to occur, additional education must be made available to patients and physicians alike to communicate when it is safe to de-escalate a patient's TKI treatment. As doctors become more familiar with the LAST study and others, such as the EURO-SKI and STIM1 studies,[16,17] and become increasingly familiar with the new, more sensitive methods to test for BCR-ABL1, new practices that allow eligible patients to discontinue TKI treatment should take hold. JoPM

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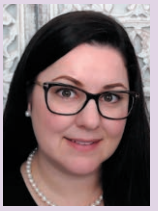
Ehab is a Professor of Medicine and Section Head of hematological malignancies in the Medical College of Wisconsin Division of Hematology and Oncology, specializing in leukemia and myelodysplastic syndromes at Froedtert Hospital. He graduated from Cairo University School of Medicine in 1994, then completed an internal medicine residency in the Cleveland Clinic Health System and a fellowship in hematology/oncology at the Karmanos Cancer Center in Detroit, Michigan, where he also served as Chief Fellow. He then went on to complete a leukemia fellowship at M.D. Anderson Cancer Center in Houston, Texas. Dr. Atallah is board certified in Internal Medicine, and Hematology. He has authored numerous publications, including journal articles, meeting abstracts, and book chapters. In 2007, he received the American Society of Clinical Oncology Foundation Merit Award. Ehab is currently the administrative director of the H Jena Khoury Cure CML consortium. He is working with several CML experts across the nation to improve the outcome of patients with CML.



Carolyn Reifsnyder

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* See, e.g., Bio-Rad website for Droplet Digital™ PCR (ddPCR™) Technology, https://www.bio-rad.com/.

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