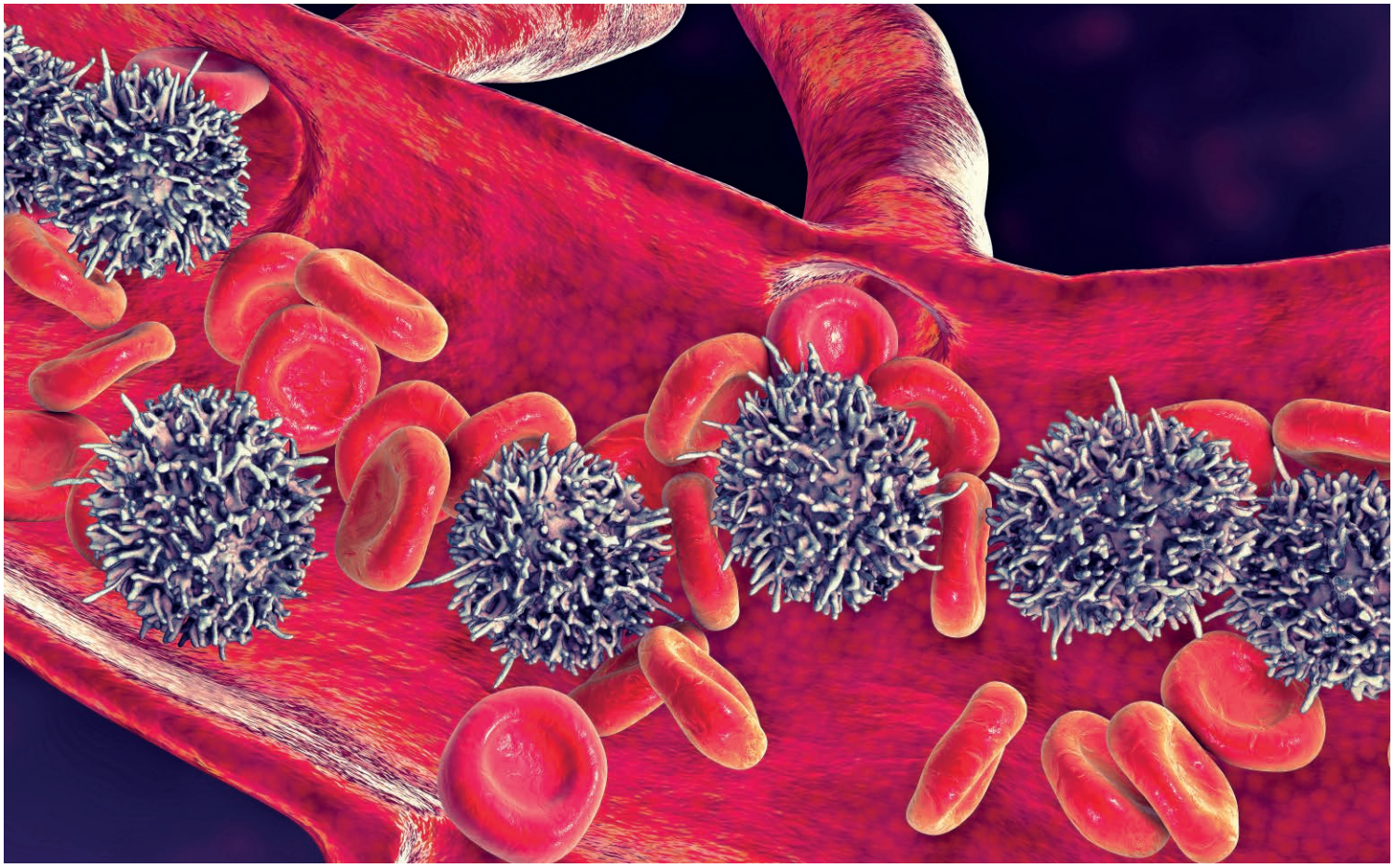


Measurable Residual Disease Detection in Hematological Malignancies: A Review from Research Tool to Clinical Diagnostic

By Christian Kazanecki, Agnieszka Seyda, Nathan Martin, Daniel Lopes de Menezes, Yixin Wang, and Sarah Hersey, Bristol Myers Squibb

Introduction

Measurable or minimal residual disease (MRD) refers to the presence of trace amounts of cancer cells that remain in a patient after initial response to intervention and could contribute to tumor recurrence. MRD detection methods have been developed to be highly sensitive and able to detect levels of residual tumor that are not detected using conventional methods (e.g., cytomorphology, medical imaging). In hematologic malignancies, bone marrow and/or blood are typical matrices collected to assess MRD. Evaluation of MRD has been an emerging and rapidly evolving field in hematologic malignancies over the past 15 years (**Figure 1**). The various use cases for MRD, especially in the context of drug development, continue to evolve with technologic improvements and an increasing body of data and experiences across each of the hematologic malignancies.

One use case of potentially great value is the use of MRD in the clinical setting to indicate response to therapy. Cytomorphologic response criteria or biochemical-based methods in hematological disorders have been found insufficiently sensitive for residual disease burden to predict, with high specificity, durable response to treatment.[1,2] Multiple studies with different therapeutic

modalities have demonstrated that while these methods can accurately identify patients that will go on to have a durable responses relative to those who will experience a disease relapse, the use of more sensitive techniques can provide more accurate prognostic information, even in patients who achieved a clinical complete remission (CR).[1,2] In contrast, a growing number of studies and meta-analyses have shown that MRD status is an effective prognostic biomarker. Patients who do not achieve MRD negativity during or after therapy are likely to relapse sooner than patients who achieve MRD negativity; furthermore, MRD negative status has been shown to be associated with superior progression-free survival (PFS) and overall survival (OS) in multiple hematologic malignancies and across multiple therapeutic modalities.[3-6] Notably, MRD remains robustly prognostic even in patients achieving deep clinical response, defined cytomorphologically or biochemically (e.g., multiple myeloma (MM) patients achieving a complete response, yet MRD positive), and highlights the importance of these sensitive measures of residual tumor burden relative to existing disease measures.

The observations that MRD assessments can provide a sensitive measure of residual disease with demonstrated clinical utility as a prognostic marker has led to renewed efforts to assess broader clinical applications. In particular, both academic and industry groups have started to implement longitudinal MRD measurements to evaluate the frequency and longer-term durability of MRD negativity. Early experience suggests this approach may be useful for guiding clinical treatment decisions and/or drug development programs. MRD could be used by drug developers as a tool for enabling more rapid and confident decision-making when assessing early therapeutic assets through comparative benchmarks. Furthermore, in many disease areas such as multiple myeloma, the standard of care therapeutics have become so efficacious that assessing new drug entities requires lengthy trials to demonstrate improved efficacy through conventional endpoints, such as Overall Survival (OS). MRD monitoring may be a potentially suitable surrogate for OS that has the possibility to enable newer, even more efficacious novel drugs to be available to patients sooner and is currently an active area of research. Groups carrying out multiple trials and consortia are working to evaluate the clinical utility of MRD, and early results using MRD to guide treatment are promising.[7-9] In this review, we will provide an overview of the evolving technologic landscape for MRD assessment across multiple hematologic malignancies; discuss the potential challenges of implementing MRD in drug development; and explore, through case studies, MRD utility from a drug development perspective. We will also discuss the effects of establishment of regulatory guidelines and greater adoption in the clinic.

Technology Landscape

Background

The assessment of MRD in hematological malignancies has evolved in part due to technological advancements in multiparametric flow cytometry (MFC), digital droplet polymerase chain reaction (ddPCR), and Next Generation Sequencing (NGS) based methods, and the parallel development of more effective therapies capable of greater disease burden reduction, leading to deeper patient response (ie, MRD negative). While the combination of assay technology and therapeutic advancements has led to a better understanding of clinically meaningful thresholds for MRD negativity, certain challenges in the measurement of MRD remain and represent a barrier for MRD to be more widely adopted as an informative assay in drug development and clinical practice. To date, there is not a single preferred MRD technology within each of the respective hematologic cancers, and it is likely that multiple approaches will continue to be explored as the field evolves, especially as some data indicate that technologies such as MFC and NGS are complementary to one another.[10] Furthermore, multiple methods may be needed because disease, therapeutic modality, and context-specific (eg, Standard of Care disease monitoring vs surrogate endpoint) features will factor into which approach may be optimal.

MRD measurement

Each MRD methodology evaluates detection of malignant/abnormal cells above or below a pre-defined cut-off, relative to the total number of cells evaluated, as a measure of the disease burden (e.g., >1 malignant cell in 100,000 total cells assayed). An exception to this is the AML NGS MRD assays, which rely on the detection of specific mutations. Peripheral blood (PB) samples and/or bone marrow aspirates (BMA) are evaluated to determine the frequency of malignant/abnormal cells; selection of the matrix is disease specific (**Table 1**). Because these methodologies are designed to determine absence of malignant cells, in contrast to detection of presence of a signal, it

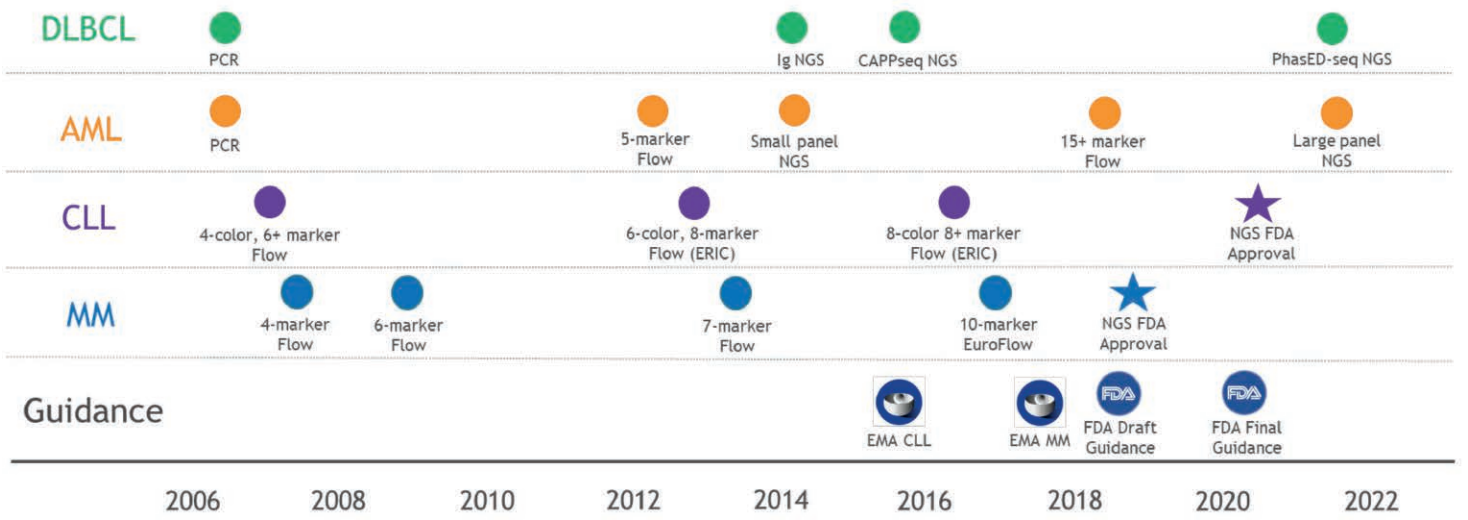


Figure 1: Timeline of evolution of MRD assessment in hematological malignancies
Select technology advancements in multi-parameter flow cytometry (MFC) and in molecular techniques have generally increased the sensitivity of MRD detection over time. The more recent development and wide use of next generation sequencing techniques, and adoption of standardized MFC panels interrogating a larger number of surface markers have generally increased the use and quality of MRD measurements.

is critical to evaluate a sufficient number of total cells from PB and/or BMA to determine MRD at clinically relevant sensitivity levels or cutoffs specified for each disease indication. This aspect is also particularly important to enable comparability and interpretability across datasets.

The primary methods to assess the MRD are based on multiparametric flow cytometry analysis of cell morphology and/or surface markers indicative of malignant cells;[11-15] and next generation sequencing analysis of B-cell or plasma cell VDJ regions to identify dominant clonality indicative of malignancy; or detection of disease-specific mutations in the case of AML.[16,17] To a lesser extent, polymerase chain reaction methods (qPCR, ddPCR) have been explored to monitor MRD.[18] These methods have advanced over the past 10-15 years, and more recently there has been a rapid improvement in the sensitivity of these methods to detect MRD, while the complexity of these assays has also increased (**Figure 1**).

Technology platforms: MFC

MFC-based methods have been the most frequently applied procedure to measure MRD, spearheaded by joint collaborative efforts for standardization and validation conducted within the framework of the European Research Initiative on CLL (ERIC) and the EuroFlow Consortium (MM and ALL).[15,19,20] MFC evaluation of MRD is mainly based on the differential phenotypic expression of cellular surface antigens between abnormal (tumor cells) and normal cells. The introduction over the years of an increasing number of antibodies in MRD panels (**Figure 1**) has allowed a more accurate definition of residual abnormal cells. MFC-based methods are advantageous in some use contexts due to the broad footprint of cytometry instruments in many clinical and third-party labs, availability of standardized staining panels, and no requirement for a pre-treatment/calibration sample to track MRD after an intervention. MFC-based methods, however, are not without limitations. MRD analysis with MFC requires manual or semi-automated gating of cell populations which need to be tailored for each patient, as the aberrant cell markers can differ between patients, and additionally, gating guides may be implemented slightly differently between labs creating potential barriers for broad standardization. Furthermore, MFC-based methods typically require analysis of fresh (i.e., not frozen or fixed) samples within 48-72 hours after collection and require a relatively higher starting number of cells to reach a desired sensitivity to account for losses from decreased cellular viability and methodological processing. While these challenges are not insurmountable, they can create additional complications for drug development use cases where standardization across global clinical trials is important to meet the high analytical validation bar that health authorities place on some biomarker use cases.

Technology platforms: NGS

While MRD in B-cell and plasma cell malignancies can be measured by PCR-based methods, this technique is quickly being replaced by a more streamlined and multiplexed methodology based on NGS. The principles behind the two techniques are very similar – this review will focus on the NGS-based methods. In both cases, specific DNA regions unique to malignant cells can be identified at diagnosis and then tracked by MRD assays designed to detect these patient-specific regions in post-treatment samples. Originally PCR was used to track patient-specific mutations (i.e., NPM1 in AML, BCR-Abl1 in CML[21,22]), but more recently malignant cells are characterized by a unique hyper-expansion of IgH gene locus derived from the combination of variable (V), diversity (D) and joining (J) gene segments rearranged during early lymphoid differentiation. The unique IGHV-IGHD-IGHJ rearrangement of each B-cell clone can be amplified using consensus primers for IGHV and IGHJ genes at the 5′ and 3′ of the rearranged region, and then used to track the presence of these unique clones, usually with the presence of a polyclonal background referable to the normal B cell population.[23,24]

In 2018, the FDA cleared the clonoseq® Assay (Adaptive Biotechnologies, Seattle, WA), an NGS-based method, for the measurement of MRD to monitor changes in burden of disease during and after treatment in MM and ALL. In 2020 the label was expanded to include CLL testing in peripheral blood and bone marrow (clonoSEQ® Assay Technical Information). Similar to the PCR-based method described above, the clonoSEQ® assay uses NGS to analyze the B-cell clonality of unique *IGHV-IGHD-IGHJ* rearrangement, without the need for allele-specific primers because the specific sequence information is captured, allowing for a standardized, accurate, and sensitive measurement of MRD well below the current MRD clinical cut-offs.

The NGS methodology does not require immediate fresh sample processing like MFC, and preservation of bio-banked samples for future analysis can be an advantage to streamline operational burden or costs for drug development use cases (e.g., in a clinical trial setting). On the other hand, NGS-based methods require a successful calibration sample (e.g., sample from diagnosis, pre-treatment sample) for clone identification (or gene mutation) to enable subsequent MRD tracking; an unsuccessful calibration results in an inability to track MRD at subsequent timepoints. Success rates of calibration have been shown to be higher in newly diagnosed populations proximal to initial diagnosis when tumor burden is highest and tumor heterogeneity is typically lower than later line patients. Therefore, successful calibration can be challenging in a subset of patients in some later line populations and/or situations where initial tumor burden is low (i.e., below 3%). Also, unlike MFC, typical NGS-based MRD assays do not have the ability to assess the hemodilution status of samples, and this must be accounted for separately, especially in the cases where preservation of samples for retrospective assessment is planned.

Several applications which need standardization still persist, notably in MRD assays for AML. While there is consensus on the core antibodies for flow cytometry assay panels,[15] individual labs have added their own markers to the panels making comparisons and meta-analyses across labs/datasets difficult. AML is unlike the other hematological diseases, due to the heterogeneity of the disease hence the AML NGS MRD assay approach relies on large mutation panels for the detection and monitoring of somatic mutations, similar to current NGS approaches for solid tumor MRD analysis (i.e., analysis of circulating tumor DNA). While there is a potential path to standardization, there is not a broadly accepted panel of mutations for this application that is both widely and consistently implemented. Also, the targeted NGS

Table 1: Primary assays for MRD detection in specific hematological malignancies

Disease	AML	MM	CLL/SLL	ALL
Sample type	BMA	BMA	PB and BMA	BMA
Clinical cut-off	10^{-3}	10^{-5}	10^{-4}	10^{-4}
Flow Cytometry method	Multiple	EuroFlow*	ERIC**	EuroFlow*
Primary NGS or PCR Assay	Multiple (mutation based, e.g., NPM1)	B-cell VDJ clonality	B-cell VDJ clonality	B-cell VDJ clonality

*EuroFlow Consortium
**ERIC – European Research Initiative on CLL
See text for abbreviations

panels utilize multiple sequencing platforms and methodologies, resulting in various sequencing depths and sensitivities.

Challenges in implementation:

Although each of the MRD testing methodologies have distinct advantages (and disadvantages), significant challenges remain for academic and industry groups to recognize the full value of MRD testing in clinical trials or disease monitoring paradigms. We summarize a few of these challenges below:

The evolution of MRD assay sensitivity and definition(s) of MRD negativity has made interpretation of historic MRD use and performance across various trials difficult. Technological improvements resulting in increased MRD depth renders comparisons with previous MRD negative results generated with a less sensitive assay difficult, and in some cases not useful in larger meta-analyses. One persistent issue is that in all cases the sensitivity, or depth of MRD negativity, of an individual patient result is ultimately dependent on the sample quality of the blood or bone marrow aspirate collected. In the case of bone marrow aspirates, obtaining high quality samples, preferably from the initial aspirates is extremely important, as hemodilution of subsequent aspirates can lead to false negative or missing results, the incidence of which can range from 5-20% or higher depending on the assay and/or trial.[25-27] The MRD assay limit of detection (LOD) is typically a "sliding scale" because the assay aims to determine absence of a signal (i.e., malignant cells) and is, therefore, dependent on sample cellularity and, specifically, the number of cells analyzed. Historically, this resulted in data from individual trials representing different definitions of MRD negativity or a mixture of MRD negative results with varying levels of sensitivity. In some cases, evaluating MRD inconsistently or at lower sensitivity levels can drastically change the prognostic value of the biomarker.[28,29]

FDA guidance on MRD

These challenges have not gone unnoticed by the FDA, which co-sponsored a number of public workshops on MRD between 2012 and 2014, resulting in the release of the guidance document "Hematologic Malignancies: Regulatory Considerations for Use of Minimal Residual Disease in Development of Drug and Biological Products for Treatment, Guidance for Industry", in draft form in October 2018 and finalized in January 2020 (see https://www.fda.gov/regulatory-information/search-fda-guidance-documents/hematologic-malignancies-regulatory-considerations-use-minimal-residual-disease-development-drug-and).[30] This guidance document attempts to drive further standardization by outlining recommendations for validation of MRD assays as well as standardization of clinical trial sample testing. For example, in order to address the challenges abovementioned, sponsors should "pre-specify the total number of events to be collected to support the quantitative assessment of MRD", and any sample that does not reach the pre-specified sensitivity (number of events), or is missing for any reason, is to be considered a non-responder (i.e., MRD positive) in the final analysis. In addition, any MRD assay used must be validated to a limit of detection 10-fold less that the threshold level for MRD reporting, setting a high bar for existing assays which are typically reported based on the maximum sensitivity attained – usually equal to the LOD of the assay.

The recommendations described in the FDA guidance are still being incorporated into validation protocols by various MRD assay providers, particularly those offering MFC-based MRD assays. The eventual effect of the guidance recommendations on the ability to have MRD included in drug labeling is still unknown. Clinical trial MRD results will be impacted by the ability to obtain high quality, non-hemodiluted samples as well as the disease and bone marrow state at the time of MRD sampling.

For NGS-based assays, this includes the ability to obtain a sample from each patient to establish the initial clonality as well as the post-treatment MRD assessment. For example, in studies where enrollment includes patients who have received prior treatment and have lower tumor burden (such as maintenance trials) the ability to determine the clonal sequence for tracking is lower at the time of screening/enrollment of patients in the trials and can significantly affect the overall MRD result and interpretation of findings. In this scenario, the lack of cellularity in these MRD samples will be reported as missing and considered non-responders (i.e., MRD positive).

Over the past few years we have seen the

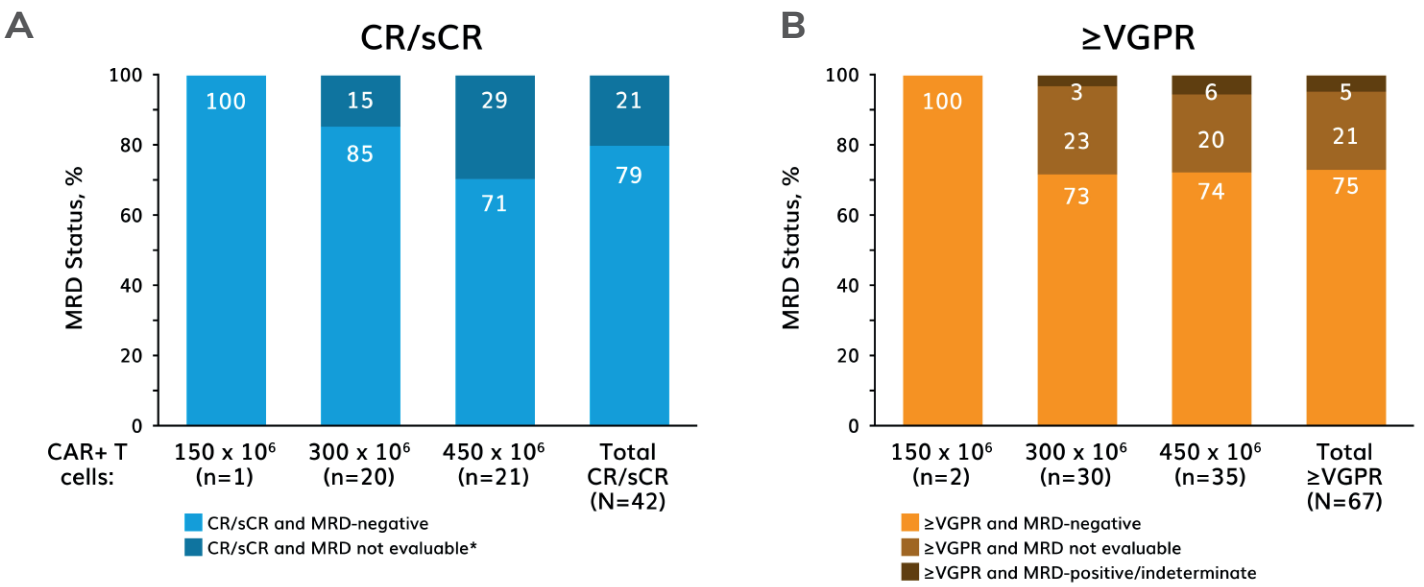


Figure 2: MRD in CR/sCR and VGPR+ patients in the KarMMa study

MRD negativity was evaluated by the clonoSeq® assay at a sensitivity of 10-5 in subjects from the KarMMa study achieving a best overall response of CR/sCR (A) or VGPR or better (B).[33] Subjects were considered MRD negative if they had an MRD negative result within 3 months or later upon achieving a best overall response of CR/sCR (A) or VGPR or better (B). *Of 42 patients with ≥CR, 8 were not evaluable for MRD and 1 had values outside the 3-mo window prior to CR/sCR.

standardization and wider adoption of both MFC and NGS assays for some indications begin to alleviate the variation resulting from assay performance differences. These advancements have primarily lead to a clearer characterization of the relationship between MRD negativity with durability of response (PFS) and overall survivial (OS) for individual disease indications, and have enabled more rigorous comparisons of response by MRD status across trials. With these advancements, questions such as which timepoint(s) are optimal to sample MRD to identify true durable responders, and whether the relationship between MRD and response will hold true for different disease settings and/or therapeutic modalities can be further interrogated.

MRD as a surrogate endpoint

The guidance released by the FDA also describes the recommendations for validation of MRD as a surrogate endpoint. These recommendations emphasized the need to conduct a large meta-analysis for each disease setting or segment to establish the relationship between the odds ratio of achieving MRD negativity and the hazard ratio for PFS/OS. The meta-analysis should take into account many aspects, some of which are: mutliple randomized clinical trials (RCTs) that demonstrate both positive and negative results; pre-specified criteria for concluding surrogacy based on assay deployed; and both trial-level and patient-level MRD and efficacy data, including pre-specified timing and window of MRD assessment; as well as discussion and statistical handling of missing MRD assessments and confounding factors.

Although numerous meta-analyses have been published that reinforce the prognostic power of MRD,[3-5] limited standardization of the definition of MRD negativity, methodologies, and the timing or window of MRD assessment with these historical studies has hampered the ability to meet FDA requirements for broad establishment of surrogacy. For example, a recent meta-analysis by Munshi[4] analyzed 43 studies and showed that MRD negativity is significantly associated with extended PFS (HR 0.35 (95% CI, 0.31-0.39); P <0.001) and OS (HR 0.48 (95% CI, 0.41-0.55); P <0.001) in a large cohort of MM patients, including both transplant-eligible and transplant-ineligible patients with newly diagnosed MM, and those with relapsed refractory MM. Hazard ratios for PFS and OS were in favor of MRD-negativity in all subgroups analyzed, including sensitivity thresholds, method of MRD, cytogenetic risk for high-risk and standard-risk cytogenetics, respectively, and disease setting.

Additionally, it must be demonstrated that the effect size observed between two or more arms in a study for achieving MRD negativity is proportional to the effect size observed for PFS and/or OS. In MM, Avet-Loiseau and colleagues[31] have shown potential feasibility for MRD to be a surrogate endpoint, whereby there was a relationship between the odds of reaching MRD negativity and hazard of PFS events. While encouraging, additional analyses are needed to satisfy the high bar outlined by regulators to establish surrogacy. In order to generate the data and analyses required, various industry and academic/industry consortiums (such as MPAACT for AML and iiTEAMM for MM), have been formed with goals such as standardizing MRD assays and their use across trials, and/or pooling trial data for the purposes of validating surrogacy in consultation with Health Authorities.

Table 2: MRD response rates with azacitidine-based treatment regimens in two different AML segments (intensive induction therapy (IC) ineligible and post IC maintenance AML patients).

MRD was reported using flow cytometric analysis of hematopoietic cell immunophenotypes. MRD was reported at a cutoff of MRD+ >10^{-3} post treatment with injectable azacitidine (AZA) or AZA + Venetoclax. For Quazar, MRD response was determined post IC/C following Placebo or Oral azacitidine (oral AZA) treatment (MRD <10^{-3}).

The VIALE-A trial evaluated the clinical efficacy of AZA plus either VEN or placebo in previously untreated patients with confirmed AML patients 75 years of age or older, who were ineligible for standard induction therapy.[34] All patients received a standard dose of azacitidine (75 mg/m2 subcutaneously or intravenously on days 1 through 7 every 28-day cycle); venetoclax (400 mg) or matching placebo was administered orally, once daily, in 28-day cycles. Study cohorts for VIALE-A consisted of 579 pts assessed for eligibility, 146 pts were excluded, 433 pts were randomized. The phase 3 QUAZAR AML-001 trial, evaluated the clinical efficacy of oral azacitidine (Oral-AZA) 300 mg) or placebo once daily for 14 days per 28-day cycle in AML maintenance setting post IC. The trial design and key inclusion and exclusion criteria have been reported in [42]. Briefly, eligible patients were aged ≥55 years, with intermediate- or poor-risk cytogenetics at diagnosis (according to the National Comprehensive Cancer Network [NCCN] 2011 AML Guidelines), had achieved first complete remission (CR) or CR with incomplete hematologic recovery (CRi) (IWG 2003 AML criteria) after intensive chemotherapy (induction ± consolidation), and were not considered candidates for hematopoietic stem cell transplant before study screening. The use and type of consolidation therapy were determined by the treating physician and occurred prior to study screening, and patients were eligible for enrollment regardless of whether or not they received consolidation. The Quazar study cohort consisted of 555 pts assessed for eligibility, 83 pts screened but not randomized, and 472 pts randomized. CR/CRi status reported at randomization for post IC (2 patients missing in the placebo arm; MRD status at randomization was missing for 2 patients in the oral AZA arm and 7 patients in the placebo arm).

	IC ineligible Previously Untreated AML (VIALE-A)		IC eligible; AML Post IC Maintenance (QUAZAR)	
Response endpoint	Injectable AZA + VEN N=286	Injectable AZA N=145	Oral AZA N=238	Placebo N=234
OS (median, months)	14.7	9.6	24.7	14.8
CR (%)	36.7	17.9	78.6	84.2
CR + CRi (%)	64.7	22.8	100	100
MRD+ at screening, n (%)	N/A	N/A	103 (43%)	116 (50%)
MRD responders, n/N (%)	67/164 (40.9%)#	11/34 (32.4%)#	38/103 (37%)	22/116 (19%)
OS: MRD+ (n)	18.7 (97)#	ND	14.6 (103)	10.4 (116)
OS: MRD- (n)	NR (67)#	ND	30.1 (133)	24.2 (111)

*Data was compiled from [34-36,42]. # MRD reported in CR + CRi subset after AZA+VEN or AZA treatment; OS (median, months) in MRD+ and MRD- segments reported in the CR + CRi subset after AZA+VEN (Pratz et al., 2002). MRD data were reported at an MRD+ cut-off of 10^{-3} using multi-parameter flow cytometry (assay details in Pratz et al., 2022; Roboz et al., 2022).

Abbreviations: CR, complete remission; CRi, CR with partial hematological count recovery; MRD, measurable residual disease; N/A, not applicable; ND, not done; NR, not reached; OS, overall survival; pts, patients.

MRD as a biomarker for drug development or treatment modification

Establishing MRD as a surrogate endpoint is important, especially in patient populations where long clinical trial durations can be signficant barriers to developing novel therapies. Challenges remain, however, and it's unlikely the path to surrogacy in heme malignancies is a straight line. In the interim, though, MRD is a useful and sensitive biomarker of tumor clearance and response kinetics that can be used in the drug development process or as a biomarker to guide treatment.

Due to the prognostic value of MRD, the utility of MRD as a biomarker to guide escallation or de-escallation is just starting to be explored in clinical trials. In this setting, MRD can be used to evaluate patients who have achieved stable MRD negativity and may be candidates for reduction in,

or cessation of, ongoing treatment with ongoing monitoring (e.g., the MASTER trial[8]).

Alternatively, MRD may be an effective biomarker to guide treatment intensification or detection of early risk of disease progression as patients either fail to reach MRD negativity after a particular regimen or timeframe, or convert to MRD positive at a timepoint before clinical progression is observed.[7] Currently, numerous trials across heme malignancies are implementing MRD as secondary or exploratory endpoints because it provides a sensitive measure of residual disease. Here we provide two examples from our own experience that exemplify not only the promise but also the complications of interpreting MRD data.

Examples using MRD as a secondary or exploratory endpoint

Example 1: MRD response as a measure for B-cell maturation antigen (BCMA)-directed CAR T-cell therapy in Relapsed and Refractory Multiple Myeloma (RRMM) patients

Idecabtagene vicleucel (ide-cel, also called bb2121) was the first approved BCMA-directed CAR T-cell therapy in late line RRMM patients. The KarMMa study (NCT03361748) evaluated MRD in patients after infusion with appropriately modified CAR T-cells.[32] MRD was evaluated at multiple timepoints post-infusion using the clonoSeq® assay at a sensitivity of 10^{-5} (1 in 100,000 cells). The primary analysis evaluated the MRD negativity rate in patients achieving a complete response (CR) or better response by IMWG criteria. Best overall response of CR/stringent CR (sCR) was achieved in 42 of 128 ide-cel treated patients. MRD was evaluable in 33 of these 42 patients. All 33 patients achieved MRD negativity, representing a MRD negative and CR/sCR rate of 26% of ide-cel treated patients[32] (see **Figure 2**). These findings demonstrated that CAR T-cell therapy (with a novel MOA in MM at the time) could drive deep, MRD negative responses in a subset of late-line patients. Of note, the CR/sCR patients who did not yield MRD results (n=9) either had missing or non-evaluable samples, and were considered MRD positive based on health authority guidelines: "For ITT-based analyses, sponsors should consider any patient without an MRD assessment as not responsive to treatment".[30]

This example highlights just one of the challenges of using MRD as an endpoint in the context of a clinical trial. For example, subsequent comparative analyses of durability of response in MRD negative vs. MRD positive CR patients could be confounded if a sufficient subset of the group of MRD positive patients (i.e., those with missing MRD results in this example) are indeed MRD negative, but placed within the MRD positive group due to the guidelines, and may motivate conducting these exploratory analyses only in evaluable patients.

Munshi and colleagues also summarized the MRD findings in patients achieving a best overall response of very good partial response (VGPR) or better (i.e., addition of VGPR patients to above summarized results in CR/sCR patients[33]). There were 25 patients with a best overall response of VGPR, and 13 of these patients achieved MRD negativity post treatment. MRD was not evaluable in 5 patients, and the remaining 7 patients were MRD positive or had an evaluable sample with an insufficient number of cells to determine MRD at 10^{-5} sensitivity level. It is less clear what MRD negativity means in these patients who have not matured to a CR/sCR response, and further analyses of these patients may provide insights into MRD assessments and dynamics after CAR T in MM patients.

Example 2: MRD response rates in intensive induction chemotherapy (IC)-ineligible and eligible AML patients with azacitidine based therapies

In AML, MRD is often evaluated using multiparameter flow cytometry at a threshold of 0.1% and cellular markers are utilized to detect leukemic cells using detection of leukemic associated phenotypes or those that are different from normal (DfN) cellular phenotypes. Depending upon the variety of cellular markers used, methods may differ in their underlying sensitivities and accuracy to detect leukemic cells, highlighting the importance of understanding the various methods and their limitations when comparing various MRD treatment data.

Table 2 presents the MRD response rates in intensive induction chemotherapy (IC)-ineligible and eligible AML patients with azacitidine based therapies compiled from the VIALE-A and QUAZAR trial data.[34,35] Generally, high remission rates can be achieved with IC with or without consolidation regimens in IC-eligible AML subjects. Yet nearly half of these IC eligible patients post IC have MRD positive disease. In the post IC setting, oral azacitidine (AZA) treatment can convert a fraction of these MRD positive patients to MRD negative state during the course of maintenance treatment, i.e., 37% (oral AZA) vs. 19% (placebo).[36] In comparison, in IC ineligible AML patients, typically older patients (>75 years), combinations of injectable AZA with Venetoclax (VEN) can achieve high 65% CR + CRi rates compared to injectable AZA monotherapy (23%). The corresponding overall MRD negativity response rates with injectable AZA and VEN compared with AZA alone were 23% versus 8% respectively.[35] Despite the high combined CR rates achieved with these treatments, MRD persists, highlighting the need for improved treatments that achieve higher MRD neg conversion rates.

These data demonstrate that comparisons of treatment regimens using MRD assessments need to be carefully considered based on the patient population and treatment setting. Furthermore meta-analyses for AML surrogacy would need to consider the elements of disease/treatment contexts associated with the study patient population and/or trial design.

In the development of more effective treatments in AML with higher CR rates, the underlying MRD data becomes important, as achievement of MRD negativity is often used to determine post remission strategies. Alternatively, monitoring of rising MRD levels may be used to develop earlier treatment strategies to circumvent AML relapse. These observations have led to increased emphasis for MRD data collection in conjunction with CR response criteria. Collection of MRD data is clearly important and comparisons with standard of care benchmarks would enable the development of improved therapeutics and/or combinations for AML.

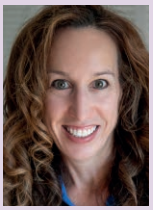
Discussion / Future Directions

The evolution of MRD testing has been characterized by increased accuracy and sensitivity through technological advancement in flow cytometric instrumentation, reagents, and high throughput sequencing methods. Health Authorities have responded by providing timely and much-needed guidances on specification of minimum requirements for validation and standardization of MRD assays for hematologic malignancies. Importantly, this guidance and oversight provides a pathway for continued technologic advancement and improvement in methodologies, while establishing the criteria for consistency in datasets to enable the critical cross-trial meta-analyses to further the utility of MRD in drug development and clinical practice.

Technological improvements in sensitivity seem to have slowed recently for a few different reasons. First, MFC may be hitting an asymptote for sensitivity given larger number of input cells required in some applications, e.g. MM Euroflow, where 10 million cells are needed to reach the established maximum sensitivity of 2×10^{-6}. In general, increasing sensitivity means increasing the total number of cells analyzed, which has become limiting based on sample characteristics and/or practical platform throughput. In our experience, a portion of samples collected do not reach the level of cellularity required for current sensitivity guidelines, and increasing this requirement will increase the percentage of

dropouts. Second, Health Authority guidances, particularly the FDA's guidance have rightfully stressed standardization and consistency in MRD testing, which may have the effect of stifling the introduction of improved assays unless they are robustly bridged to current/prior assays. This need to standardize and bridge can be particularly challenging when fresh BM and/or patient samples are needed to conduct these experiments. In addition, the requirement of a large meta-analysis of multiple trials with consistent MRD testing to establish surrogacy may focus MRD testing on existing assays for the medium term, with the resulting benefits of increased standardization and comparability/poolability across studies. Assuming surrogacy is eventually established, the result of establishment may be a wider adoption of existing assays, which further raises the bar for adoption of new assays.

For the reasons stated above, future technological improvements to MRD may not be focused on increasing sensitivity of current assays, but rather on shifting to a matrix that can be accessed less invasively (i.e., shift from BM to PB). Such advancements may result in increased adoption as well as more frequent testing to better characterize the timecourse for MRD with a given intervention. These advances are an active area of research currently, and initial approaches, such as in CLL, are screening in blood first, followed by confirmation in bone marrow by an established technique. This two-step approach may be developed in other disease applications, such as AML or DLBCL, where NGS can be used to monitor the presence of specific disease-associated mutations in blood using ctDNA. Another example is in the use of Mass Spectrometry for MRD in peripheral blood in MM, which is increasing in use as evidenced by recent publications.[37-40] Other newer technologies such as single cell analysis that combines both molecular and protein marker based techniques,[41] will greatly increase the usefulness of MRD for R&D applications, such as developing a greater



Sarah Hersey

Sarah is currently the Vice President, of Translational Sciences & Diagnostics for Bristol Myers Squibb. She has more than 20 years of industry experience in leading R&D organizations, CLIA and GLP laboratories, device manufacturing, consortiums, assessment and implementation of technology strategies, and development of in vitro diagnostics. In addition, she continuously works to further education and awareness in the Precision Medicine and Companion Diagnostic spaces. Prior to joining BMS, Sarah was the Vice President, Precision Medicine & Companion Diagnostics for Celgene, where she founded their Precision Medicine Organization in 2016. She joined Celgene after having held the position as the Global Head of Future Precision Medicine for Novartis, where the team submitted and gained approval for multiple Health Authority applications; including the first pre-market approval for a distributable NGS CDx.

Sarah has also held multiple roles with increasing responsibility within Johnson and Johnson (JNJ) including Global Head of Operations, Assay Program Leader for Companion Diagnostics Center of Excellence, Director of Materials Process Development and Enabling Technologies. At JNJ, Sarah co-led or was the operating company's representative on key corporate strategic initiatives including, but not limited to: Nanotechnology, Critical to Quality Flow Down and Pathology Business Opportunity Team and had accountability for CLIA laboratories in the US and Europe. In addition, her prior experience also includes start-up and establishment of a GLP laboratory, technical support and quality assurance. Sarah has BS and MS degrees in Chemistry and Biology from Northern Illinois University, Regulatory Affairs Certification (RAC) and an MBA from California State University.

Daniel Lopes de Menezes

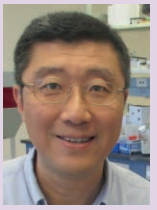
Daniel Lopes de Menezes, Ph.D. is Senior Director of Hematology Translational Medicine at Bristol Myers Squibb. He is the translational medicine asset lead for Vidaza® and Onureg® in myeloid cancers and was an integral member of the development team of ONUREG®.

Dr Menezes' career spans more than 20 years of experience in the biotechnology and pharmaceutical industry at Chiron Corporation, Novartis Institutes of Biomedical Research, Celgene and Bristol Myers Squibb, leading target discovery, pharmacology and translational research in early and late-stage oncology drug development. Dr Menezes joined Bristol Myers Squibb through the Celgene acquisition having served scientific roles with increasing responsibility, including leadership and accountability of non-clinical and translational research on various portfolio projects. Prior to Celgene and Bristol Myers Squibb, Dr Menezes spent his early biotech career at Chiron and Novartis as a lab head responsible for the pharmacology strategy on multiple small molecule inhibitors, antibody and antibody drug conjugate (ADC) programs.

Dr Menezes received his Ph.D. in Pharmacology from the Faculty of Medicine, University of Alberta, Canada. He trained at the British Columbia Cancer Agency in Vancouver, Canada as a Terry Fox/NCIC postdoctoral fellow.

Christian Kazanecki

Christian Kazanecki has over 17 years of experience in various roles within the medical device/diagnostic and pharmaceutical industries, including discovery research/IP, assay design, development, and validation, and CLIA lab operations. He is currently Director, Translational Sciences and Diagnostics, in the Bristol Myers Squibb Translational Medicine organization where he drives the strategic implementation of companion and complementary diagnostics (CDx) biomarkers in support of the BMS hematology portfolio. Prior to joining BMS, Christian worked at Johnson & Johnson and Roche Molecular Systems Inc., where he was responsible for diagnostic assay design, development, transfer and validation of several molecular assays to support clinical trials and biomarker research. Christian received his Ph.D. in Microbiology & Molecular Genetics from Rutgers University.



Yixin Wang

Yixin Wang is Executive Director of Diagnostic Sciences for Hematology, Immunology, Cardiovascular diseases at Bristol Myers Squibb. Yixin has 25-year of experience in research & development of diagnostic assays and products. Prior to joining BMS, he worked at Celgene, Celsee Diagnostics (now Bio-Rad), Ventana Medical Systems of Roche, and Veridex of Johnson & Johnson. Yixin has extensive expertise on development of cancer diagnostic products. He has also worked with pharmaceutical groups to identify biomarkers, as well as develop, validate and commercialize companion diagnostic assays.

Yixin received his Ph.D. in Molecular Biology from Cornell University and his B.S. in Biochemistry from Beijing University. He has published 72 peer-reviewed articles. He serves as scientific reviewer on NCI and DOD study sessions on cancer detection, diagnosis and therapeutics.

Nathan Martin

Nate is a Senior Principal Scientist, Translational Research within the Immuno-oncology and Cell Therapy Thematic Research Center at BMS, where he leads the translational biomarker activities for the ide-cel program. Dr. Martin has previously held translational research positions at BioMarin Pharmaceuticals and Flagship Biosciences, and received his PhD from the University of California – Los Angeles

Agnieszka Seyda

Agnes Seyda is currently a Director of Translational Sciences and Diagnostics, at Bristol Myers Squibb where she leads development and implementation of regulated assays for patient selection, stratification, or monitoring in support of BMS hematology and immunology portfolios. She has more than 20 years of industry experience. Prior to joining BMS, she held multiple roles with increasing responsibility within Celgene and Janssen Pharmaceuticals. Her prior experience also includes various R&D roles in the Medical Devices sector within Ethicon and DePuy. Agnes received her B.Sc. degree in Biochemistry from McMaster University in Canada and a Ph.D. degree in Biochemistry from University of Toronto, Canada. She is a co-inventor on over 50 patents.

understanding of the evolution of the disease and mechanisms of resistance, as was recently demonstrated in AML.

Over the past decade and half, a tremendous amount of data has been collected providing objective evidence of the value of MRD as a prognostic marker in multiple hematological diseases. During this same timeframe, great strides have been made in increasing the relapse-free and overall survival rates, creating multiple needs for MRD use in drug development including: 1) the need for a biomarker with high prognostic value to gain an early perspective on responses with novel agents or treatment paradigms in early phase clinical studies, and 2) a need to establish surrogacy using MRD to decrease overall clinical trial duration and bring new therapies to patients in a reasonable timeframe, especially disease settings where long trial times could be a potential barrier to development. There are multiple examples, including the two provided in this review, where MRD has value to improve the interpretation of response observed with novel agents in clinical trials. To bring MRD across the finish line as a surrogate endpoint, however, we must continue to focus on successfully implementing MRD assays/techniques that meet the minimum requirements outlined by health authorities, establish the appropriate window(s) to evaluate MRD negativity post-intervention, take into account differences in reponse kinetics within different disease settings and treatment modalities, and establish a method(s) for monitoring hemodilution for some assays where this is not already evaluated during sample analysis (e.g., NGS). As the field of MRD testing evolves, there will be a continued need for balance between ongoing technology improvements and assay standardization, as well as settling on regulatory mechanisms to enable comparisons between established and newer, more advanced technologies, in consultation with the Health Authorities. JoPM

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