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Review

Time to repeal and replace response criteria for acute myeloid leukemia?

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ABSTRACT

The International Working Group (IWG) response criteria for acute myeloid leukemia, published in 2003, have remained the standard by which the efficacy of new drugs is measured in clinical trials. Over the last decade, concepts related to treatment response have been challenged by several factors; for example, the dissociation between early clinical response and survival outcome in older patients, the recognition that epigenetic and newer differentiating-agent therapies may produce delayed responses and also hematologic improvement/transfusion independence without a morphologic response, and evidence that remissions without minimal (or measurable) residual disease (MRD) may result in outcomes superior to those of morphologic remissions with persistent MRD. The evolving role of MRD status as a potential surrogate for predicting long-term survival has enhanced the clinical need to standardize and incorporate emerging technologies that enable deeper responses beyond those recognized by the IWG, and to pre-emptively identify patients at risk of early relapse. The potential for therapeutic interventions to erase MRD and alter the natural history represents an important and open research question. Reviewed here are some of the implications and challenges associated with establishing and incorporating new treatment response criteria, initially into clinical research, and eventually into real-world practice.

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by a diverse spectrum of mutated genes and a multi-clonal genomic architecture comprising preleukemic and leukemic clones that evolve dynamically over time and under the selective pressure of therapy [1,2]. Induction chemotherapy with cytarabine and an anthracycline ("7 + 3") has been the standard of care for more than 4 decades for patients with newly diagnosed AML [3]. Until recently, few new therapeutic advances have been made. In 2017, however, the FDA approved four drugs for AML – midostaurin, enasidenib, CPX-351, and gemtuzumab ozogamicin (GO) [4]. The therapeutic landscape is therefore starting to change from a "one-size-fits-all" 7 + 3 approach to AML treatment, to one that is more tailored to the biological characteristics of the patient's disease.

Despite the evolving therapeutic landscape, the standard criteria for assessing response has remained relatively unchanged since the International Working Group (IWG) response definitions for AML were published in 2003 [5]. Furthermore, the primary endpoint for drug registration has historically been dependent on showing improvements in overall survival (OS). The recent full approval of enasidenib has, however, revealed the FDA's receptiveness to approving transformative therapies in areas of high unmet need based on phase 2 data, and on endpoints other than OS. Since the IWG criteria were published, concepts related to treatment response have been challenged by a variety of factors - the dissociation between response and long-term survival with hypomethylating agents (HMAs), the variable relationship between IWG-defined response criteria and survival of older patients with AML receiving novel therapies, and recognition that HMAs or differentiation therapies have delayed kinetics of response compared with

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chemotherapy. Moreover, the evolving impact of measurable residual disease (MRD) status as a potential surrogate for long-term survival [6,7] has strengthened the clinical need to incorporate emerging technologies to enable deeper response assessments beyond those recognized by the IWG [8].

The recent 2017 update to the European LeukemiaNet (ELN) recommendations for diagnosis and management of AML acknowledged several of these changes, and proposed new working criteria to facilitate response assessments in clinical trials [8]. Reviewed here are some of the implications and challenges arising from current IWG response criteria, and incorporation of new ELN response criteria into clinical research and into real-world practice.

2. Complete remission

The primary goal of AML treatment is to attain a cure, at least for younger patients. For older patients who may not tolerate curative therapies, the therapeutic goal may be to prolong survival while maintaining an improved quality of life. The currently established early surrogate of long-term survival is the attainment of morphologic complete remission (CR), which, according to IWG 2003 criteria [5] requires < 5% myeloblasts in bone marrow, the absence of circulating blasts, hematologic recovery (as evidenced by a peripheral blood absolute neutrophil count (ANC) > 1000 cells/μL and platelet count > 100,000/μL, with no need for red blood cell (RBC) transfusions), and the absence of extramedullary disease. An analysis of 6283 patients with newly diagnosed AML showed that at least 94% of patients alive 5 years after receiving treatment had initially achieved CR [9], consistent with the notion that long-term OS in AML is CR-dependent, and that CR is an important predictor of OS. The possibility that a patient with AML is cured can be considered when there is no further excess mortality compared with that of the general population [10]. For patients treated with intensive chemotherapy, this generally requires a sustained CR of at least 5 years, with cure proportions varying from ~50% for adults up to the age of 40, to < 5% for patients over the age of 70 years [10,11], despite CR rates > 80% for younger patients and 40–60% for patients over age 60 years [8,12,13]. Thus, while CR is an important predictor of OS, relapse after achieving CR is a progressively common fate with increasing age, indicating that morphologic assessment is inadequate as the sole predictor of long-term survival [14].

Many clinical trials include CR with incomplete hematologic recovery (CRi; < 5% bone marrow blasts with residual neutropenia [ANC < 1000 cells/μL] or thrombocytopenia [platelets < 100,000/μL]), or CR with incomplete platelet recovery (CRp) as part of a composite response endpoint in clinical trials. The ELN 2017 recommendations incorporate CRi into response criteria. Retrospective studies have indicated that relapse-free survival (RFS) and OS outcomes for patients achieving either CRi or CRp are inferior to those of patients who achieve CR, while still superior to those of patients who do not attain any response [7,9]. Patients who attain CRi or CRp are, not surprisingly, more likely to have detectable MRD [7]. Another criterion included in the ELN 2017 recommendations is "morphologic leukemia-free state" (MLFS), which refers to morphologic bone marrow blast clearance to < 5% in a marrow sample in which at least 200 cells are enumerated or cellularity is ≥ 10%, in the absence of blasts with Auer rods or extramedullary disease, and with no hematologic recovery required [8]. MLFS is generally used as an additional measure of clinical response in the context of clinical trials. An additional term, not included in the ELN 2017 recommendations, is CR with partial hematologic recovery (CRh), defined as < 5% blasts in the bone marrow, no evidence of disease, and partial recovery of peripheral blood counts (platelets > 50×10^9/L and ANC > 0.5×10^9/L). While used in a registration trial for acute lymphoblastic leukemia [15], and in the prescribing information for enasidenib (recently approved for treatment of relapsed or refractory AML), but not currently recognized in the IWG 2003 or the ELN 2017 criteria, the term CRh aims to describe marrow blast clearance and evidence of partial hematologic recovery not captured by current CR, CRi, or CRp criteria [16]. In clinical practice, once there has been adequate hematologic recovery, it may be preferable to initiate post-remission therapy in an attempt to maintain dose intensity, rather than delay treatment in order to wait for full count recovery.

The use of the term 'primary refractory AML' is also problematic. The number of standard induction chemotherapy cycles delivered to patients with AML varies internationally, with some centers and cooperative groups routinely administering two induction cycles, and others only one. In some centers, therefore, the definition of 'primary refractory disease' requires failure of only one induction cycle, whereas in other centers, failure to respond to two sequential induction chemotherapy cycles is required for this designation. A one- vs. two-cycle induction therapy approach will therefore influence when patients are considered to be 'primary refractory,' and referred for salvage therapy or to a clinical trial. An analysis by the Eastern Cooperative Oncology Group (ECOG) [17], and a subsequent analysis by the Southwestern Oncology Group (SWOG) [18], showed no difference in survival outcome for patients requiring one vs. two induction cycles to achieve CR, in contrast to the widely accepted perception that the requirement for two chemotherapy courses to achieve CR ranks with other poor prognostic features, such as adverse karyotype [18]. In contrast to the findings by SWOG, a recent study by the UK National Cancer Research Institute (NCRI) included 802 patients who did not attain CR after a first induction chemotherapy course, with 204 of these patients achieving a CR or partial remission (PR) only after the second induction course [19]. The 5-year survival for patients who attained CR after the first induction course was 40%, significantly better than that for patients attaining CR after the second induction course (23%; hazard ratio [HR] 1.39 [95% confidence interval (CI), 1.15–1.69]; $P = 0.0008$ [19]. Cumulative CR rates should therefore indicate whether a one- or two-cycle induction strategy was used, and the cumulative response rates after completing one or two induction cycles should be reported routinely in clinical trial publications. Survival appears particularly poor if more than two induction cycles are needed to achieve CR [20]. As a result, the ELN defined primary refractory AML as failure to achieve a CR or CRi after two courses of intensive induction chemotherapy, excluding patients with death in aplasia or death due to an indeterminate cause [8]. Such patients would be considered reasonable candidates for experimental therapies. Going forward, clinical studies aiming to recruit 'primary refractory' patients should consider using this consensus definition.

A confounding issue in identifying 'primary refractory AML' is that the assessment of post-chemotherapy blast counts may be confused by marrow regeneration, making it difficult to distinguish morphologically between persistent disease and true remission. Interobserver variation may also result in variable reporting of outcomes for patients with borderline levels of bone marrow blasts after therapy. Multiparameter flow cytometry (MFC) may help to distinguish between marrow regeneration and persistent disease [21]. A study found that 67% of pediatric patients classified morphologically to have persistent disease (5–15% blasts) actually had no detectable MRD by MFC, highlighting the potential risks of making treatment decisions based on morphology alone [21]. Another study, conducted in patients with AML aged ≤ 55 years showed that patients with a morphologically defined partial response (PR; 5%–15% blasts, or < 5% blasts with hypocellular marrow [5]) after the first cycle of chemotherapy, had a comparable 5-year survival rate (44%) to patients who achieved CR (53%) [22]. Similarly, a recent study found that among 104 patients failing to attain CR or CRi after one cycle of intensive chemotherapy, 38% had a PR at the post-treatment assessment [23]. Survival outcomes for those attaining PR was superior to patients not attaining PR (median OS not reached vs. 7.4 months, respectively; $P = 0.002$) [23]. It is likely, therefore, that more accurate and objective measures of clinical response, as provided by newer MRD technologies, will play an

increasingly important role in response assessment criteria in the future.

3. Moving beyond CR: detection of minimal/measurable residual disease (MRD)

Given the prognostic significance of MRD, the 2017 ELN recommendations for response criteria now recognize CR without MRD (CR_{MRD-}) as a defined response endpoint in AML [8]. MRD assessment will likely complement baseline patient risk assessment factors such as age, karyotype, and a panel of molecular mutations defined by the ELN 2017 recommendations (including *FLT3*-ITD and mutations affecting *NPM1*, *CEBPA*, *TP53*, *ASXL1*, and *RUNX1*) [8,24] in determining patient prognosis. The ELN 2017 recommendations propose that MRD should be monitored following induction and consolidation courses to assess remission status and the kinetics of disease response, and then in surveillance after consolidation, to detect impending morphologic relapse [8]. Currently, the most commonly used methods of MRD assessment are MFC, and real-time quantitative polymerase chain reaction (RT-qPCR) of specific mutations or gene fusions detected at initial diagnosis [25–29]. MRD monitoring for patients with acute promyelocytic leukemia (APL) has become standard and will not be a subject of this review [30]. Recently, the ELN MRD Working Party has published consensus criteria regarding the use, performance, and reporting of flow cytometric and molecular methods for MRD determination in AML [31].

3.1. Multiparameter flow cytometry

MFC techniques are based on the expression of antigens that characterize diverse lineages of hematopoietic cells. AML blasts may either be defined as expressing distinct leukemia-associated immunophenotypes (LAIPs), or by manifesting an immunophenotypic maturation profile detected using a fixed antibody panel that is "different from normal" [28,32]. In general, LAIPs delineate markers or combinations of markers that are aberrantly expressed in AML [33]. MFC may not be informative if there has been a phenotypic shift over time (false negative), while the "different from normal" method must avoid reporting of pre-leukemic or dysplastic cell populations as MRD (false positive).

A retrospective analysis of 245 adults aged 18 to 80 years with AML who achieved CR, CRp, or CRi with intensive or lower-intensity treatments, examined the relationship between MRD, as detected by 10-color MFC, and clinical outcomes [7]. Any measurable level of residual disease was considered MRD-positivity. MRD was detected in 19%, 54%, and 61% of patients achieving CR, CRp or CRi, respectively, suggesting an association between the extent of hematologic blood count recovery and presence of sub-microscopic disease burden [7]. That CRp and CRi were associated with MRD more frequently, was true regardless of treatment intensity or whether patients had newly diagnosed or relapsed/refractory AML. The cumulative incidence of relapse at two years was ~80% among patients who achieved CR/CRp/CRi but were MRD positive, compared with only ~35% for patients who were MRD negative ($P < 0.001$; Fig. 1A). A significant difference in estimated OS was also demonstrated between these groups ($P < 0.001$) (Fig. 1B) [7]. Although pretreatment covariates such as cytogenetics, monosomal karyotype, relapsed/refractory vs. newly diagnosed AML, and presence of *FLT3*-ITD were significantly ($P < 0.05$) associated with relapse in univariate analyses, only MRD status, type of response (CR, CRi, or CRp), monosomal karyotype, and allogeneic stem cell transplant (alloSCT) remained significantly associated with relapse in multivariable comparisons [7]. Indeed, patients who did not achieve a CR_{MRD-} had a > 90% probability of relapse if they did not receive alloSCT [6]. Even among patients who did receive alloSCT, relapse rates in those with detectable MRD while in CR or CRi were high. In a study of 88 patients in CR or CRi before transplant, 2-year relapse rate post-

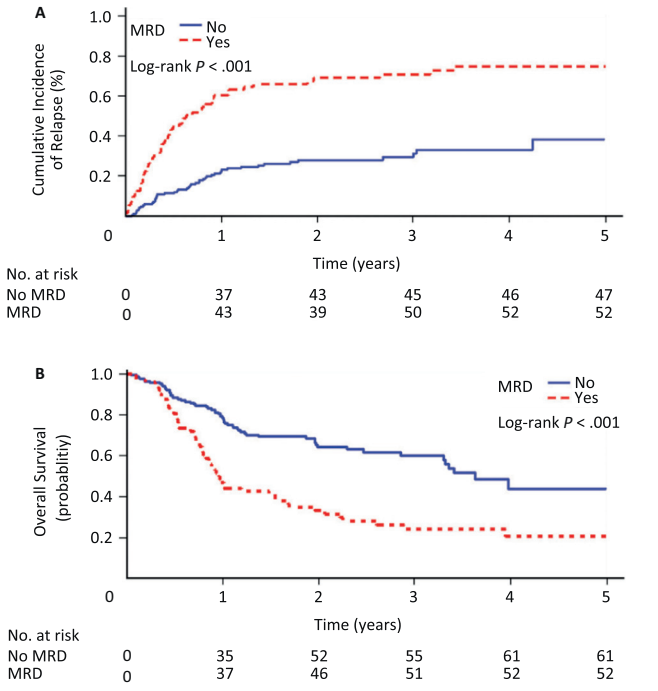


Fig. 1. Impact of minimal residual disease (MRD) after induction therapy on outcomes in patients with acute myeloid leukemia. (A) Cumulative incidence of relapse. (B) Kaplan-Meier survival analysis of probability of overall survival. Originally published by the American Society of Clinical Oncology. [Chen et al: J Clin Oncol 33(11), 2015: 1258-64].

transplant was ~64% in patients with MRD detected before transplant, compared with 18% among patients without MRD before transplant [34].

Similarly, the SWOG S0106 study in younger AML patients (ages 18–60 years) found that at the time of CR, 22% of patients had detectable MRD using MFC [35]. Those who required two cycles of induction therapy to achieve CR were more likely to be MRD-positive than were patients who obtained CR after the first chemotherapy cycle (41% vs. 20%, $P = 0.05$). Patients in CR with detectable MRD had significantly shorter OS (HR 2.32 [95%CI, 1.42–3.77]; $P < 0.001$), RFS (HR 2.28 [95%CI, 1.45–3.60]; $P < 0.001$), and higher relapse risk (HR 2.17 [95%CI, 1.27–3.70]; $P = 0.005$) [35]. MRD, therefore, is highly informative for risk assessment among patients who achieve morphologic CR, and will assist in identifying those for whom post-remission interventions may be considered. Consensus criteria to guide clinical decisions for AML patients based on MRD status have been proposed by an ELN Working Party [31]. This ELN group recommended a cut-off of 0.1% as the threshold to distinguish MRD-positive from MRD-"negative" patients. However, it was noted that MRD levels below 0.1% may still be consistent with residual leukemia. Further analyses to examine the clinical relevance of MRD levels below 0.1% are therefore needed. Multiple factors must be considered when standardizing MRD detection by MFC, including the sample source (bone marrow or peripheral blood), sample processing variables, fluorochrome selection, antigen selection, the number of events to be acquired (a sensitivity of 0.01% necessitates 1,000,000 events), gating strategies, and variation in how results are interpreted and reported [29]. At diagnosis, numerous aberrant antigenic phenotypes may be observed, complicating the selection of the most suitable markers of MRD [29]. Although centralized laboratory testing is desirable for MRD monitoring by MFC, efforts to implement such centralized testing may be logistically challenging.

Attempts to complement the detection of MRD by MFC include techniques to quantify $CD34^+CD38^-CD123^+$ leukemic stem cell (LSC) populations using fluorochrome-conjugated isotype antibodies and

isotypic controls of the corresponding fluorochromes. A single 8-color tube was recently developed with the goal of distinguishing hematopoietic stem cells (HSCs) from LSC populations and identifying chemoresistant LSC populations that can be quantified, tracked during treatment, and correlated with outcomes [36,37]. The LSC detection tube, which is planned to become commercially available, includes antibodies to CD45RA, CD123, CD33, CD38, CD44, CD45 and a marker cocktail comprising CLL-1, TIM-3, CD7, CD11b, CD22 and CD56. The method has the potential to be adopted as an international standard for clinical validation in prospective AML trials. Although it is widely accepted that MRD is strongly associated with clinical relapse, an important question remains whether therapeutic intervention for a patient who is MRD-positive will improve clinical outcome compared with intervention at the time of morphologic relapse. MRD analysis was incorporated prospectively into the HOVON 132 AML/SAKK study in intermediate-risk AML, in which patients with detectable MRD were considered to be at poor-risk and were recommended to undergo alloSCT [33]. Additionally, the UK NCRI is conducting a prospective 'monitor' versus 'no monitor' clinical trial to evaluate the effect of MRD techniques on influencing clinical practice, and ultimately, patient outcomes (trial identifier ISRCTN55675535). The results of both of these prospective studies are eagerly awaited.

Although MFC has been criticized for its lack of standardization and reproducibility, morphologic enumeration of marrow blast percent is not entirely reproducible either. Using the ELN's recommendation for examination of 500 cells [8], disagreement on identification of only 10 cells can convert a report of 6% blasts (relapse) to 4% blasts (no relapse). A repeat marrow examination may not address the problem, given the heterogeneity of the marrow as an organ. Even ignoring disagreement on identification of individual cells, 95% confidence intervals for 20/500 blasts (4%) and 30/500 blasts (6%) overlap: 2–6% and 4–8% [38]. In contrast, MFC evaluates several orders of magnitude more cells than does morphology, conferring smaller confidence intervals and greater sensitivity to MRD assessment.

3.2. RT-qPCR

RT-qPCR is used to measure molecular mutations and leukemia-associated fusion transcripts in a quantitative manner relative to a suitable "housekeeper" gene, usually *ABL* [25–29]. RT-qPCR has high sensitivity, with a detection range of 10^{-4} to 10^{-6} cells [39]. A rigorous approach to standardizing RT-qPCR assays was undertaken by the Europe Against Cancer (EAC) program, which established a framework for development and validation of common protocols for performance of RT-qPCR assays among an international network of expert laboratories [40]. A key advantage of RT-qPCR is the quantification of the independent housekeeping gene performed in parallel with the target under investigation, thereby enabling identification of suboptimal samples that may contribute to false-negative results. A potential limitation of this method however, is that not all patients have a detectable marker available for molecular tracking. Furthermore, it is important to identify molecular markers that are stable at relapse and are not associated with pre-leukemic biology, which may give rise to false negative and positive associations with relapse, respectively.

The role of ongoing MRD monitoring in AML has been best studied in several large-cohort core binding factor (CBF) leukemia studies [41,42]. For example, in the UK MRC AML15 trial study, blood and bone marrow samples were requested at diagnosis, after each course of chemotherapy, and thereafter every 3 months in the first year of follow-up, every 4 months in the second year, at 6-month intervals in the third year, and at relapse. The presence of > 50 copies of *CBFB-MYH11* MRD by RT-qPCR in the bone marrow after the third course of chemotherapy was found in 17/53 (32%) patients with inv.(16) and was linked to a clinical relapse risk of 100% [42]. Similarly, the presence of *RUNX1-RUNX1T1* MRD with > 500 copies in bone marrow was found in 15/91 (16.5%) patients with t(8;21) AML, and was also linked to a 100% relapse risk [42]. In patients with t(8;21), the median time to relapse from detection of MRD in bone marrow was 4.9 months, and in peripheral blood was 4.5 months; for inv.(16) patients, the median time to relapse from detection of MRD was 3 months for both peripheral blood and bone marrow, with rising MRD levels on serial monitoring almost universally associated with hematologic relapse [42].

The relationship between MRD positivity and relapse may be stronger than that between MRD and survival. For example, in the UK MRC AML15 study, patients with CBF-AML who received fludarabine, cytarabine, idarubicin and G-CSF (FLAG-Ida) had a greater log reduction in MRD than did patients receiving 7 + 3 induction chemotherapy with or without etoposide, but this difference did not lead to a better OS for those patients [42], possibly due to the efficacy of salvage strategies in CBF leukemias. Consistent with this, in a wider AML population, Lambert and colleagues evaluated the relationship between levels of *NPM1* gene transcripts assessed using complementary DNA-based RT-qPCR in 77 patients with an *NPM1* mutation at diagnosis in the randomized ALFA-0701 trial of GO [43]. While *NPM1* MRD (defined as > 0.1% in bone marrow) after induction and at the end of treatment predicted a higher risk of relapse (HR 3.66 [95%CI, 1.10–12.15]; $P = 0.035$), MRD positivity was not correlated with OS (HR 3.06 [95%CI, 0.71–13.24]) [43].

3.3. Molecular markers of MRD

Among 1540 patients with AML enrolled in prospective trials of intensive chemotherapy, Papaemmanuil et al. identified more than 5000 driver mutations across 76 genes or genomic regions [2]. Despite the large variety of mutations in AML amenable to quantitative molecular tracking, to date only a fraction of this genetic diversity has been validated for clinical MRD use. Some molecular mutations, particularly in methylation-regulating genes, may represent clonal hematopoiesis of indeterminate potential (CHIP). Jaiswal et al. analyzed the peripheral blood of 17,182 individuals who did not have a diagnosed hematologic malignancy [44]. Mutations associated with hematologic malignancies were observed in 11.7% of people aged 80–89 years and in 18.4% of people aged 90 years or older, most commonly *DNMT3A*, *TET2*, and *ASXL1* [44]. Therefore, there is a higher likelihood that clonal abnormalities representing pre-leukemic biology will be detected in progressively older patient populations after intensive chemotherapy. Consistent with this notion, mutant *DNMT3A* persists in 40–50% of patients after chemotherapy [45]. Despite this, such patients can remain in long-term remission. In a study by Pløen et al., 14 patients (31%) with detectable *DNMT3A* mutations after chemotherapy remained in remission for up to 8 years post-diagnosis [46]. Similarly, mutations in *IDH2* may persist in differentiated maturing cells of patients treated with the mutant *IDH2* inhibitor, enasidenib, such that patients can attain CR and remain in remission with no reduction in mutant *IDH2* burden [47]. *TET2* mutations can also persist after induction chemotherapy during morphological remission [48]. Conversely, some mutations may be associated with subclonal populations (e.g. *FLT3*-ITD and *RAS*) that may be effectively eradicated by chemotherapy and are no longer present at the time of relapse [25]. Krönke et al. investigated clonal evolution in the bone marrow or peripheral blood at diagnosis of 53 patients aged 24 to 66 years with mutant-*NPM1* AML [49]. They performed molecular analyses for 10 AML-associated gene mutations that frequently co-occur with mutant-*NPM1* (e.g., *FLT3*-ITD, *DNMT3A*) in addition to high-resolution, genome-wide, single-nucleotide polymorphism (SNP) array profiling. At the time of relapse, gene mutation patterns had changed from those at diagnosis in 34 patients (64%), including 5 patients who no longer carried the *NPM1* mutation. The most common mutations acquired at relapse were in *ETV6*, *TP53*, *WT1*, *NF1*, and *FLT3* genes [49].

NPM1 is the most commonly mutated gene in AML, occurring in ~30% of all patients and present in up to 50% of patients with normal karyotype [2,50-52]. *NPM1* appears to be a primary pathogenetic

mutation and a highly expressed transcript in AML, making it ideal for RT-qPCR techniques, which have a sensitivity of up to 1 in 10^6 for the detection of mutant alleles [49,53,54]. A study by Ivey and colleagues showed that of 346 patients with *NPM1*-mutated AML and normal karyotype, 150 patient subgroups could be identified based on co-mutational profiles [55]. Despite this mutational complexity and heterogeneity, *NPM1* mutations remained present at relapse in 69/70 cases, demonstrating the utility of this marker as a stable measure of MRD [55]. After completion of two courses of chemotherapy, 30/194 patients (15%) had detectable MRD in peripheral blood, which preceded impending clinical relapse in 86% [55].

The value of mutant-*NPM1* for MRD detection was supported by a French study that evaluated 229 previously untreated patients with *NPM1*-mutated AML [56]. Patients failing to achieve a 4-log reduction in peripheral blood MRD at first remission after induction chemotherapy had a higher chance of relapse at 3 years than did patients achieving a 4- to 5-log reduction in MRD (66% [95%CI 46–84] vs. 21% [12%–33%], respectively), and also had a reduced 3-year survival rate (41% [95%CI 21%–60%] vs. 91% [69%–98%]) [56]. This study also showed that patients with a < 4 log reduction in mutant-*NPM1* variant allele frequency (VAF) during first CR had improved outcomes if they received alloSCT, whereas patients with a > 4 log reduction in MRD did not benefit from transplantation.

The ELN MRD Working Party recommends that MRD analysis be performed at 3-month intervals for at least two years if patients have a validated MRD marker, including mutant *NPM1* or fusion genes such as *RUNX1-RUNX1T1*, *CBFB-MYH11* or *PML-RARA* [31]. In this recommendation, complete molecular remission (CR_{MRD-}) is defined as two successive MRD negative samples over a period of ≥ 4 weeks at a sensitivity level of at least 1 in 1000. A proposed definition of molecular progression in patients with persistent low copy number disease is an increase of MRD copy numbers ≥ 1 log10 between two positive samples separated by at least 4 weeks. Molecular relapse, in contrast, defines patients converting from an MRD-negative to MRD-positive state, with a ≥ 4-week interval increase in MRD of ≥ 1 log10 [31].

3.4. Next-generation sequencing for MRD monitoring

There has been a shift away from automated ('first-generation') Sanger sequencing for genome analysis toward 'next-generation' sequencing (NGS), which allows multiple molecular biomarkers to be monitored simultaneously [57]. Relevant AML/myeloid panels are commercially available (e.g., SureSeq myPanel™ NGS Custom AML Panel, Oxford Gene Technology, Begbroke, Oxfordshire, UK; LeukoVantage Myeloid Neoplasm Mutation Panel, Quest Diagnostics, Madison, NJ, USA; TruSight® Myeloid Sequencing Panel, Illumina, San Diego, CA; and Human Myeloid Neoplasms Panel, Qiagen, Venlo, The Netherlands). NGS platforms involve template preparation, sequencing and imaging, and data analysis protocols that vary among instrument manufacturers.

Klco et al. used NGS (xGen® AML Cancer Panel v1.0, Integrated DNA Technologies, Coralville, IA, USA) to evaluate samples from patients with AML at diagnosis and after treatment with standard induction chemotherapy [48]. Post-treatment mutational profiling of patients in morphologic CR on day 30 revealed persistence of AML clones defined by a VAF of ≥ 2.5% after induction chemotherapy in 24/50 (48%) cases [48]. The persistence of mutations was associated with significantly shorter event-free survival (EFS) (6.0 vs. 17.9 months; HR 3.67 [95%CI, 1.93–7.11]; $P < 0.001$), compared with patients with undetectable residual mutations, independent of cytogenetic risk. A lower VAF threshold of ≥ 1.0% for detectable mutations at day 30 was also associated with an EFS of 7.9 months (95%CI, 4.5–9.9) vs. 25.6 months (95%CI, 11.4, not reached) for patients lacking lesions above this threshold (HR 3.33 [95%CI, 1.61–6.89]; $P = 0.001$) [48]. It should be noted that at present there is no clear definition of MRD negativity.

More recently, Jongen-Lavrencic et al. presented preliminary data from patients participating in HOVON/SAAK clinical trials, including 482 younger patients (age < 65 years) who had received two induction chemotherapy cycles and consolidation [58]. In 430 patients (89%), somatic driver mutations were present at diagnosis; these patients were separated into a training cohort (n = 283) and a validation cohort (n = 147). Persisting mutations, predominantly in *DNMT3A*, *TET2*, and *ASXL1* ("DTA"), were detected in 51% of patients in morphologic CR. In the training cohort, these mutations did not predict relapse at any VAF threshold. Rather, they merely appeared to reflect clonal hematopoiesis (similar to findings in older patients by Jaiswal et al., above [44]). In contrast, when any additional persistent mutation was present together with one of these mutations in the training cohort, there was a significant correlation with relapse (5-year cumulative incidence of relapse 76.4% vs. 39.4%, $P = 0.002$). Moreover, any non-DTA MRD was associated with an increased risk of relapse (subdistribution HR [SHR] 1.85 [95%CI, 1.27–2.70]; $P = 0.001$). This finding was confirmed in the validation set. In addition, NGS MRD was predictive of poorer survival in both cohorts. In multivariate analysis, adjusting for age, white blood cell (WBC) count, ELN 2017 risk, and number of induction cycles needed to achieve CR, NGS MRD had independent prognostic relevance for both relapse (HR 1.89 [95%CI, 1.34–2.65]; $P < 0.001$) and poorer OS (HR 1.64 [95%CI, 1.18–2.27]; $P = 0.003$) [58].

4. Limitations to the use of CR as a surrogate of survival

4.1. Dissociation between CR and OS in elderly AML trials

The UK NCRI AML16 study in older patients with AML (median age 74 years) examined outcomes for 406 patients randomized to receive either clofarabine or low-dose cytarabine (LDAC) [59]. Although clofarabine induced a significantly higher rate of CR (22%) than did LDAC (12%; HR 0.47 [95%CI, 0.28–0.79]; $P = 0.005$) and also of overall response (CR + CRi; 38% vs. 19%, respectively; HR 0.41 [95%CI, 0.26–0.62]; $P < 0.001$), these significantly higher response rates did not translate into improved survival (2-year survival rates were 12% vs. 13%, respectively, HR 0.96 [95%CI, 0.78–1.19]; $P = 0.7$) [59]. To explain this outcome, it was noted that 60-day mortality rate was somewhat higher in the clofarabine arm (32%, vs. 26% with LDAC), and that 2-year survival rate in patients who did not attain CR was significantly worse for those receiving clofarabine than for comparable patients in the LDAC arm ($P = 0.02$) [59].

In another study by the same group, the combination of GO plus LDAC was compared with LDAC alone in 495 older patients with AML (median ages 76 and 75 years, respectively) [60]. The overall response rate (CR + CRi) with the combination regimen was almost double that of LDAC alone (30% vs. 17%, respectively; odds ratio 0.48 95%CI, 0.32–0.73]; $P = 0.0006$). However, no differences in RFS (31% vs. 40%, respectively; HR 1.11 [95%CI, 0.73–1.67]; $P = 0.6$), or 1-year survival (25% vs. 27%; HR 0.99 [95%CI, 0.83–1.16]; $P = 0.9$), were observed between the GO-+LDAC and LDAC monotherapy groups [60], again suggesting that achievement of CR/CRi may not be a reliable early surrogate of prolonged OS, especially among elderly patients with AML.

Preliminary data presented for the combination of LDAC plus volasertib vs. LDAC monotherapy in a large ($N = 666$) phase 3, placebo-controlled, randomized trial in patients aged ≥ 65 years with untreated AML who were not eligible for intensive therapy, showed the overall response rate (CR + CRi) with the LDAC + volasertib combination regimen to be 25.2%, compared with 16.8% with LDAC + placebo ($P = 0.071$). The trend for higher overall response with LDAC + volasertib was not associated with a similar trend for improved median OS, which was 4.8 months with the combination regimen vs. 6.5 months for LDAC alone (HR 1.26 [95%CI, 0.95–1.67]; $P = 0.113$) [61]. Again, early response was not an accurate surrogate for long-term OS.

Similar examples are reported for the HMA, azacitidine. Van der Helm et al. compared azacitidine with standard 7 + 3 cytarabine-anthracycline induction in patients with newly diagnosed AML aged ≥ 60 years [62]. CR or PR was achieved in 73% of patients treated with intensive chemotherapy but in only 42% of patients treated with azacitidine ($P = 0.005$). Despite the higher response rate with intensive chemotherapy, 1-year survival was 57% vs. 56% with azacitidine alone ($P = 0.93$), and 2-year survival was 35% in both groups ($P = 0.92$) [62]. Moreover, azacitidine treatment was associated with significantly fewer days in hospital and reduced transfusion requirements compared with intensive treatment [62].

Comparable findings emerged from a study comparing response rates of patients with newly diagnosed AML who received standard chemotherapy ($n = 84$) with 83 matched older patients (≥ 60 years) receiving HMAs (69 patients received decitabine and 14 received azacitidine) [63]. Although overall response rate was superior with standard chemotherapy compared to decitabine-based treatment (50% vs. 28%, respectively; $P < 0.01$), as was CR rate (43% vs. 20%; $P < 0.01$), induction chemotherapy failed to significantly reduce death compared with the HMAs (HR 0.76 [95%CI, 0.49–1.17]; $P = 0.21$) [63].

Older patients aged ≥ 60 years are more likely to have prior hematologic disorders or treatment-related AML, and are more likely to harbor a higher frequency of poor risk karyotypes, compared with patients aged < 60 years [64], as well as poorer-risk mutations affecting *ASXL1* (15% vs. 8%, respectively; $P < 0.05$), *RUNX1* (23% vs. 9%; $P < 0.001$), and *TP53* (14% vs. 6%; $P < 0.01$) [65,66]. The frequency of favorable-risk genotypes, such as mutant-*NPM1* AML are also lower in older patients (22% in patients aged ≥ 65 years vs. 34% in patients < 60 years) [65,66]. Adverse disease biology in older patients may thus limit the influence of treatment on survival, despite an initial response. Although achievement of CR/CRi may not be as reliable an indicator of long-term survival than has been the experience among younger patient populations, it would be interesting for future studies to examine whether CR_{MRD-} has better utility as a surrogate of survival in these older patient populations, with the potential to provide a meaningful endpoint for early phase studies seeking to identify promising drugs suitable for larger registration studies.

4.2. Stable disease in clinical trials

IWG response criteria were designed to assess response to chemotherapy and have limitations when applied to assessment of newer therapeutic modalities, such as HMAs and drugs that induce cellular differentiation, for which the kinetics of response may be slower, and improvements in hematologic function, and even survival, may occur in the absence of bone marrow blast clearance [67–69].

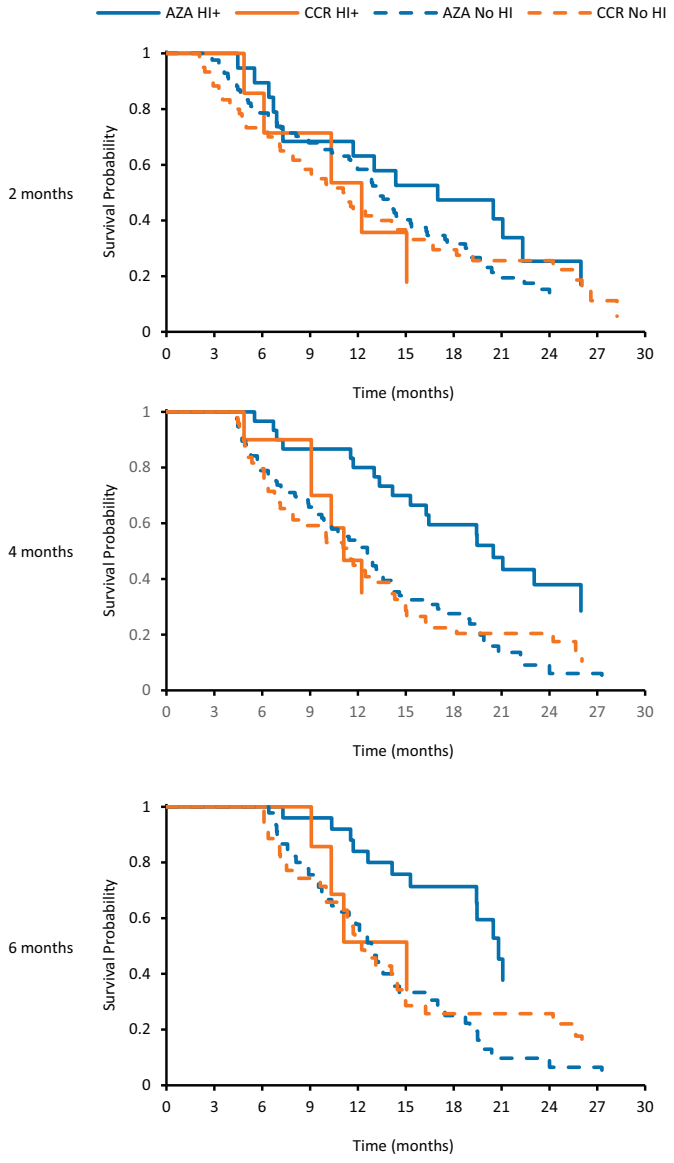
Mutations affecting *IDH1* and *IDH2* occur in ~10–20% of patients with AML, with frequency increased in older (≥ 65 years) compared with younger patients (< 60 years) (28% vs. 18%, respectively) [65,66]. Selective, small-molecule inhibitors of mutant IDH1 and IDH2 proteins are currently in clinical trials, including several mutant-IDH1 inhibitors (ivosidenib [AG-120], IDH305, FT-2102) and AG-881, which purportedly inhibits both mutant IDH1 and IDH2 proteins. Enasidenib (AG-221) is the first targeted IDH inhibitor to receive regulatory approval in the United States; it is indicated for treatment of adult patients with relapsed/refractory AML harboring an *IDH2* mutation [16]. In the phase 1 dose-escalation and expansion portions of a phase 1/2 trial, enasidenib monotherapy was associated with an overall response rate (CR + CRi + PR + MLFS) of 40.3% among patients with mutant-*IDH2* relapsed/refractory AML [70]. For patients who attained CR, 20.6%, 67.6% and 82.4% did so by cycles 3, 5 and 7, respectively [70]. Notably, mutant-*IDH2* VAF can remain unchanged in patients achieving remission during enasidenib treatment, consistent with its mechanism of action of inducing responses by promoting differentiation and maturation of leukemic blasts, resulting in neutrophils which retain the *IDH2* mutation [47,71].

In the enasidenib study, OS for non-CR responders (13.8 months [95%CI, 8.3, 17.0]) was superior to that of those who did not achieve a response (7.0 months [5.0, 8.3]), suggesting that clinical responses less than CR may be relevant in some patients [70]. Moreover, preliminary data show that some patients maintaining stable disease (SD) during the first 90 days of enasidenib treatment can achieve CR at a later timepoint [72]. An initial increase in percentage of bone marrow blasts or an absolute increase in blood blasts (often associated with leukocytosis) may be observed during early treatment with mutant-IDH inhibitors [8] and should not necessarily lead to discontinuation of therapy. Rather, addition of hydroxyurea could be considered to mitigate complications associated with hyperleukocytosis. In the absence of disease progression or unacceptable toxicity, enasidenib administration for up to 5 months appears to be necessary to capture the majority of clinically responding patients [16,72].

Analogous to the mutant IDH inhibitors, up to 4–6 treatment cycles may be required to attain a clinical response to HMAs [73,74]. First responses to azacitidine have been reported as late as cycle 16 of therapy [69,75]. Hematologic improvement (HI), as defined by the IWG 2006 criteria for myelodysplastic syndromes (MDS) [76] in the absence of a conventionally-defined bone marrow response during treatment with a HMA may potentially be beneficial to patients with AML, as measured by reduced transfusion burden and improved quality of life, as well as prolonged OS compared to no attainment of HI [5,67-69,75,77-80]. Of 214 patients treated with azacitidine in the Austrian Azacitidine Registry, those who attained any HI had a median OS of 16.1 months vs. 4.5 months for patients who did not ($P < 0.001$), and attainment of HI during treatment was independently predictive of improved OS in multivariate analysis, while bone marrow response was not [75]. The international, phase 3, randomized AZA-AML-001 trial included older patients (age ≥ 65 years) with newly diagnosed AML (> 30% bone marrow blasts) who received azacitidine (n = 241) or a conventional care regimen (CCR; n = 247), either 7 + 3 induction chemotherapy, LDAC, or best supportive care only [81]. Median OS for all patients in the AML-001 trial was 10.4 vs. 6.5 months with azacitidine vs. CCR, respectively [81]. A post hoc analysis evaluated the OS of patients in this study who maintained SD (defined as failure to achieve a response but not meeting criteria for disease progression over a period of more than 8 weeks), with or without achieving HI as defined by the IWG 2006 criteria for MDS [76,82] (Fig. 2). Patients treated with azacitidine with sustained SD at 6 months who attained HI had a 7.9 month improvement in median OS (20.8 vs. 12.9 months for azacitidine-treated patients without HI) [82], whereas improvement in median OS in CCR-treated patients with SD at 6 months who attained HI was only 2.9 months (15.1 vs. 12.2 months for CCR patients with or without HI, respectively) [82].

Transfusion independence during HMA treatment has also been associated with improved OS for AML patients who do not attain IWG-defined CR [78,83,84]. In a post hoc analysis of the international, phase 3 DACO-016 study of decitabine vs. 'treatment choice' (LDAC or supportive care only) in 485 older patients (age ≥ 65 years) with newly diagnosed AML, median OS among patients failing to attain CR was 9.8 months for patients receiving decitabine who were RBC transfusion independent at baseline or during treatment, vs. 6.4 months for patients who were transfusion-dependent at baseline or on-study (HR 0.67 [95%CI, 0.47–0.98]; $P = 0.0221$) [84].

Similar to what is observed with mutant-IDH inhibitors, hematologic responses to HMAs may be delayed, making it important for physicians to not prematurely terminate treatment in patients with SD or lack of overt disease progression. In recognition of the need to provide guidance on what would constitute disease progression for the purpose of clinical trials, the ELN has proposed a set of three criteria to define progressive disease for patients receiving novel therapies [8]. These include 1) a > 50% increase in bone marrow blasts from baseline, with an absolute blast increase of ≥ 15% if the baseline blast count is < 30% at baseline; 2) persistence of > 70% bone marrow



*HI must have started on or before the landmark date and lasted until after the landmark date.

Fig. 2. Overall survival with azacitidine at 2-month, 4-month, and 6-month landmarks, in patients with stable disease at each landmark, with or without hematologic improvement*.

blasts for at least 3 months without any evidence of hematologic improvement, defined as either (a) a doubling in either neutrophil count to an absolute level > 0.5×10^9/L [500/μL], and/or platelet count to > 50×10^9/L [50,000/μL] non-transfused, and/or (b) > 50% increase in peripheral blood blasts not related to differentiation syndrome; and 3) emergence of new extramedullary disease [8]. It is hoped these criteria may be useful for characterizing patient benefit not linked to bone marrow blast reductions, as has been observed with differentiating-agent and HMA therapies.

5. EFS potential as an alternative to OS as a primary endpoint in AML evaluation trials

AML is a rare disease and the increased partitioning of this uncommon cancer into biomarker-linked subgroups with approved therapies, such as APL, mutant *FLT3*, and mutant *IDH*, make it increasingly difficult to successfully recruit patients for clinical trials within a meaningful timeframe to achieve adequately powered improvements in OS. There is a great need for more efficient and flexible trial methodologies, such as Bayesian and adaptive trial designs, to be acceptable for drug registration purposes. Furthermore, reliable early surrogates of survival as a primary endpoint for AML studies are critical to accelerate clinical development timelines in AML. The recently conducted SWOG S1203 study randomizing patients to standard 7 + 3 chemotherapy versus idarubicin with high-dose cytarabine, with or without vorinostat, utilized EFS as a primary outcome measure. In this study, EFS (events were defined as failure to attain CR, relapse, or death) closely paralleled RFS and OS, suggesting EFS was a robust marker of drug efficacy [85]. In contrast, a similar frontline German study randomizing the addition of sorafenib or placebo to standard induction chemotherapy also utilized EFS as the primary study endpoint [86]; despite a significant increase in median and 3-year EFS and RFS rates with sorafenib, there was no difference in OS outcomes between sorafenib and placebo. The explanation for this discrepancy was not clear, but was speculatively considered to relate to inferior response to salvage approaches or alloSCT in the sorafenib arm. In the phase 3 ALFA-0701 study comparing standard chemotherapy alone versus standard chemotherapy in combination with GO, EFS was the primary endpoint and OS was a secondary endpoint [87]. EFS and OS were both statistically superior with the combination regimen compared with standard therapy alone, but in an exploratory analysis that adjusted for baseline covariates (age, WBC count, and CD33 expression on blast cells), the GO + chemotherapy combination regimen remained associated with significantly longer EFS (HR 0.61 [95%CI, 0.45–0.82]; $P = 0.001$) but the OS difference became non-significant (HR 0.66; [0.41–1.04]; $P = 0.07$). Nevertheless, as an aggregate measure of remission attainment, duration of remission, and OS, improved EFS represents a meaningful endpoint for patients and physicians that is likely to be increasingly used as a more rapid outcome measure in comparative AML outcome trials. The use of EFS rather than OS as a primary study endpoint may also eliminate the potentially confounding effects of post-relapse salvage therapy and “outside-of-study crossover” on OS, and the inclusion of relapse as a component of EFS acknowledges the significant impact relapse has on patient quality of life and physical integrity. The inclusion of CR_{MRD-} into AML response criteria also provides the opportunity to explore whether newer technologies can augment the accuracy of EFS correlations with OS in future studies. The ELN 2017 recommendations suggest a standardized clinical trial reporting template to encourage a consistent approach to publication of results, thereby enabling physicians and patients to more easily interpret outcomes among different studies.

6. Summary

This paper has attempted to outline the evolving landscape of how AML treatment is evaluated, highlighting the many challenges that have underscored the need for change. It is inherently clear that methods used to evaluate AML therapies need to be tailored to both the biological mechanism of action of the drug in question and to the patient population under study. The definition of treatment failure for a particular drug must therefore take into consideration the kinetics of expected response and the impact of the drug on hematologic improvement in the absence of morphologic blast reductions. The shortcomings of morphological CR as an early response endpoint are starting to make way for more sensitive and clone-specific techniques to enable more accurate measures of submicroscopic levels of disease. These technologies must now cross the experimental threshold and find their way into routine clinical practice to allow physicians access to more accurate methods of relapse prediction. Validation of MRD as a meaningful parameter for initiating therapeutic intervention, and acceptance by regulatory authorities of CR_{MRD-} as a relevant clinical endpoint for therapeutic evaluation remain key future challenges for AML stakeholders. The methodology used for MRD assessment remains heterogeneous across centers and countries, both with respect to antibody

panels and evaluation of marker kinetics in flow-based MRD measurement, and regarding relevant markers and thresholds for molecular MRD detection. Efforts to harmonize and standardize MRD methodology internationally are essential. The quality and cost of survival are also important metrics relevant to both patients and their physicians. Measurement of EFS represents one way of incorporating factors beyond survival into response evaluation, as well as shortening timelines for clinical trial completion. Consistency in how trial outcomes are reported would be beneficial.

The ELN 2017 recommendations have incorporated several of these challenges into the first major revision of response assessment in AML for over a decade. Greater consistency in how patients are defined for inclusion into refractory AML studies, and adoption of new criteria defining treatment response and progression, should provide us with more harmonized and relevant measures of new drug activity for incorporation into clinical trials, and ultimately, routine clinical practice.

Practice Points

- Concepts related to treatment response, as defined in the 2003 International Working Group response criteria for AML, are being challenged by more recent advances in technology and newer drugs with novel mechanisms of action.
- Early morphologic response outcomes may not be an accurate surrogate of long-term survival in older AML patients receiving lower-intensity treatment regimens.
- Differentiating agents and lower-intensity therapies may produce delayed responses; continued treatment in the absence of disease progression or unacceptable toxicity may be required to capture these responses.
- Hematologic improvement and transfusion independence without a morphologic response may confer clinical benefits.
- Remissions without MRD are superior to morphologic remissions with persistent MRD; emerging technologies to more accurately determine the risk of relapse are likely to become incorporated in future clinical trials and standard-of-care practice.

Research Agenda

- Limitations and pitfalls of current AML response criteria
- Rationale and potential for newer response criteria detailed in the ELN 2017 AML recommendations

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