Minimal residual disease–directed therapy in acute myeloid leukemia

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Case presentations

Case 1: A 35-year-old man with a normal white blood cell (WBC) count (9.3 × 10^9/L) was diagnosed with acute myeloid leukemia (AML) with a t(8;21)(q22;q22) translocation in 25/25 metaphases. The RUNX1-RUNX1T1 fusion gene was detected by real-time quantitative polymerase chain reaction (PCR), whereas studies for mutations involving KIT and FLT3 were negative. After 1 cycle of induction therapy with cytarabine/idarubicin according to the "7+3" schema, he achieved a morphologic complete remission (CR) with a 2-log reduction of RUNX1-RUNX1T1 transcript levels. The patient has an excellent performance status and no comorbidities. Should you recommend allogeneic hematopoietic cell transplantation (HCT)?

Case 2: A 43-year-old woman was diagnosed with cytogenetically normal AML; molecular studies for gene mutations involving NPM1, CEBPA, and FLT3 were negative. After standard induction chemotherapy, she achieved a morphologic CR and then underwent 1 cycle of consolidation therapy with high-dose cytarabine. During the pre-HCT work-up in anticipation of matched related donor transplant, she is found to have evidence of minimal residual disease (MRD) by multiparameter flow cytometry (MFC); no prior MFC studies are available. She has no comorbidities other than arterial hypertension, and her performance status is excellent. Are you recommending additional cycle(s) of chemotherapy to attempt MRD eradication before HCT?

Introduction

In recent years, several methods have been developed to detect submicroscopic MRD in AML patients in morphologic remission.[1-4] The existence of small numbers of leukemic cells among normal hematopoietic cells can be identified based on numeric or structural chromosomal changes, gene mutations, antigen receptor rearrangements, abnormal gene expression, altered cell growth, and immunophenotypic abnormalities. Thus far, most exploited for MRD detection and quantification in AML are MFC- and PCR-based approaches, which can achieve sensitivities up to 10^{-5} to 10^{-6}.[1-5] MFC has gained popularity for the detection of MRD in AML because it can be applied to the vast majority of AML patients, although the identification of immunophenotypic abnormalities can be challenging, especially if a diagnostic specimen is not available or the disease has evolved over time. PCR-based approaches are typically limited to specific patient subsets, but recent methodologic advances (eg, based on next generation sequencing or digital PCR) allow leukemia-associated mutations to be tracked more comprehensively, thereby broadening the scope of molecular MRD detection (defined in this study as the detection of chimeric fusion genes, somatic mutations, or aberrant gene expression).

For several reasons, including variations in health care provision and laboratory infrastructures between countries and, perhaps, the fluidity with which MRD detection methodologies are evolving, implementation of standardized MRD assessments into clinical practice has remained a major challenge. Nevertheless, increasing evidence indicates that the presence of MRD, measured either molecularly (as in case 1) or by MFC (as in case 2), identifies patients at particularly high risk of relapse and provides powerful prognostic information beyond pretreatment characteristics, such as cytogenetic or molecular abnormalities.[4] This observation has sparked interest in risk-adapted treatment strategies that are based on the MRD status to improve patient outcomes. Herein, we examined the evidence supporting such an approach.

Literature search strategy

A systematic literature search, restricted to humans and the English language, was conducted using MEDLINE (October 24, 2014), Embase (October 31, 2014), and CENTRAL (Cochrane Register of Controlled Trials; November 10, 2014) (see supplemental Tables A1 and A2, available on the *Blood* Web site). Three authors reviewed all abstracts. Studies were included if they provided useful extractable data for AML (other than acute promyelocytic leukemia [APL]) for which MRD parameters were used to direct therapy. Potential unpublished articles were also sought by searching Web of Science, the Web sites for the conference proceedings from the American Society of Hematology (2012-2014) and the American Society of Clinical Oncology (2012-2014), as well as the Clinical Trial Registry (www.clinicaltrials.gov; November 10, 2014). Recommendations were developed based on the Grading of Recommendations Assessment Development and Evaluation system (Table 1).[6]

Search results and discussion

Our systematic literature search resulted in 603 records after duplicates had been removed (MEDLINE, n = 236; Embase, n = 402; and

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Table 1. Summary of GRADE recommendations on rating the strength of recommendations and quality of evidence

Strength of recommendation		Quality of evidence	
1 ("Strong")	Desirable effects of an intervention clearly outweigh (or clearly do not outweigh) the undesirable effects	A ("High")	Further research is very unlikely to change our confidence in the estimate of effect
2 ("Weak")	Trade-offs between desirable and undesirable effects are less certain (eg, because of low-quality evidence or evidence suggesting closely balanced effects)	B ("Moderate")	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate
		C ("Low")	Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate
		D ("Very low")	Any estimate of effect is very uncertain

GRADE, Grading of Recommendations Assessment Development and Evaluation.
Each recommendation consists of a numerical score denoting the strength of the recommendation and a letter denoting the quality of the evidence.[6]

CENTRAL, n = 16). Of these, 28 were reviewed in full. No randomized, controlled study was found to address MRD-directed therapy for non-APL AML.

Case 1: MRD-directed therapy of favorable-risk AML

Patients with t(8;21)(q22;q22) AML generally have a relatively favorable prognosis. With intensive induction chemotherapy, nearly all individuals who do not die of treatment-related toxicities will achieve a morphologic CR, and with repeated courses of intensive consolidation therapy, the relapse risk may not exceed 20% to 35% in 3 to 5 years.[7-11] Consequently, these patients have, on average, no survival advantage with allogeneic HCT while in first remission because the transplant-related mortality is greater than the decrease in relapse rates afforded by the transplant.[12,13] However, significant heterogeneity within t(8;21)(q22;q22) leukemias is widely appreciated. Several variables associated with worse outcome have been recognized in at least some studies, including a high WBC count and the presence of *KIT* or *FLT3* mutations at diagnosis.[7,14-19] Recent studies have highlighted this disease heterogeneity by identifying subsets of patients with distinct risks of disease recurrence based on the degree of reduction in *RUNX1-RUNX1T1* transcripts.[9-11,20-23] Specifically, in the largest study conducted to date on 163 patients, a >3 log reduction in transcript burden after the first course of induction therapy and a >4 log reduction after the first course of postremission therapy were associated with cumulative incidences of relapse (CIR) of only 4% and 13%, respectively; in this study, the clinicians were blinded to the MRD results, which thus did not influence patient management.[9] Other studies came to qualitatively similar conclusions.[10,22,23] By comparison, other series, including one on 116 patients, have suggested that MRD levels after induction have no prognostic relevance while they are informative after consolidation therapy.[11,20] Direct comparison of these studies is hindered by the fact that different real-time quantitative PCR methodologies and data normalizations were employed, the timing of MRD assessment varied, definitions for the quality of MRD response differed, and variable chemotherapy regimens were used. Moreover, because of their retrospective nature without independent prospective confirmation, estimates from these studies may be subject to significant bias. Nonetheless, the available evidence suggests that optimal outcomes are achieved when patients with t(8;21)(q22;q22) AML obtain either a molecular remission or very significant reductions in *RUNX1-RUNX1T1* transcripts with induction and postremission therapy; higher-intensity regimens may lead to deeper log reductions after the first course of chemotherapy.[9,24] Emerging evidence from a study by Jourdan et al also suggests that information from posttreatment *RUNX1-RUNX1T1* transcript levels may be preferable over high WBC or *KIT*/*FLT3* mutational status to identify patients with high-risk t(8;21)(q22;q22) AML, as only MRD but not the other factors was of significant prognostic impact in multivariate analyses.[25]

No randomized trial has so far tested whether patients with t(8;21)(q22;q22) AML who are in morphologic CR with suboptimal reduction in *RUNX1-RUNX1T1* transcript levels would benefit from allogeneic HCT. However, a recent multicenter study suggests the potential value of such an approach.[11] This study examined 116 patients aged 15 to 60 years with t(8;21)(q22;q22) AML who achieved morphologic remission with 1 to 2 courses of induction therapy according to the "7+3" schema, and then completed 2 cycles of consolidation therapy consisting of intermediate-dose cytarabine (1 to 2 g/m^2 every 12 hours for 3 days) with or without an anthracycline. The lack of a major molecular response (defined as a >3 log reduction in *RUNX1-RUNX1T1* transcript levels from baseline) after the second course of consolidation, or loss of major molecular response within 6 months, was used to categorize patients into high- and low-risk. High-risk patients were recommended to proceed to myeloablative allogeneic HCT, whereas low-risk patients were advised to undergo 6 additional cycles of chemotherapy (intermediate-dose cytarabine for cycles 3 and 4, then cytarabine 100 mg/m^2 for 7 days in combination with an anthracycline [cycle 5], homoharringtonine [cycle 6], mitoxantrone [cycle 7], or aclamycin [cycle 8]); autologous HCT was permitted after 4 courses of consolidation. Sixty-nine of the 116 patients (59%) were compliant with this risk-adapted approach (with 40/69 high-risk patients undergoing allogeneic HCT and 29/47 low-risk patients receiving chemotherapy); the remaining patients served as non-risk adapted controls. Overall, the risk-adapted treatment approach resulted in survival outcomes similar to what has been reported earlier. In additional "as-treated" landmark analyses, allogeneic HCT was associated with a lower relapse rate and better survival, as compared with chemotherapy in high-risk patients (5-year CIR: 22.1% vs 78.9%, $P < .0001$; 5-year disease-free survival [DFS]: 61.7% vs 19.6%, $P = .001$; 5-year overall survival [OS]: 71.6% vs 26.7%, $P = .007$). Conversely, low-risk patients did not significantly benefit from allografting with regard to CIR (14.7% vs 5.3%, $P = .33$) and even had inferior DFS relative to those treated with chemotherapy/autologous HCT (70.3% vs 94.7%, $P = .024$).[11] However, because of the possibility of significant bias in the above analyses, the benefit of allogeneic HCT for high-risk patients with t(8;21)(q22;q22) AML needs to be confirmed in further, better controlled studies; if large enough, such studies could also assess which role different transplant conditionings and donor sources might play for high-risk t(8;21)(q22;q22) AML. Although some patients with insufficiently reduced transcript levels can remain relapse-free even without allogeneic HCT, others will experience disease recurrence even when transplanted, therefore a more aggressive therapy (like allogeneic HCT) is not always associated with a better outcome in these patients.[11] Future investigations will also need to carefully revisit the impact on OS, given

that some studies with careful long-term follow-up data indicate that many patients with favorable-risk AML can be salvaged after first disease recurrence.[7,26]

Recommendation. For an adult with t(8;21)(q22;q22) AML who has achieved morphologic CR with persistence of *RUNX1-RUNX1T1* transcripts with 1 course of induction therapy, no data exists to advocate the immediate use of allogeneic HCT. We suggest monitoring *RUNX1-RUNX1T1* transcript levels and considering allogeneic HCT if a >3 log reduction in *RUNX1-RUNX1T1* transcript levels from baseline is not reached after the 2nd course of consolidation or lost within 6 months (grade 2C). The optimal definition of what should be considered insufficient reduction in *RUNX1-RUNX1T1* transcripts levels and the best timing of this assessment might change based on future data.

Case 2: MRD-directed therapy of intermediate-risk AML

Unlike favorable-risk patients, those with intermediate-risk disease based on revised Medical Research Council/National Cancer Research Institute (NCRI) or European LeukemiaNet criteria have generally been considered appropriate candidates for allogeneic HCT in first morphologic CR, particularly if comorbidity scores are low and an HLA-matched donor is available.[12,13] This recommendation has recently been challenged by analyses from the Medical Research Council/NCRI suggesting that equivalent OS in this risk group may be achievable by delaying transplantation until after the first relapse.[26] Even if an allogeneic HCT is performed in first morphologic CR, posttransplant relapse remains a substantial risk. Several retrospective studies have suggested that, on average, standard cytarabine-based consolidation chemotherapy before allogeneic HCT for AML patients of all risk groups in first morphologic CR does not improve posttransplant outcomes.[27-30] Unfortunately, in all these trials, information on MRD was not available, and it is unknown whether additional postremission therapy could benefit a subset of patients with MRD. Numerous studies have convincingly demonstrated that MRD before allogeneic HCT is independently associated with a significantly increased risk of subsequent relapse and inferior survival.[1,5] This relationship would justify risk-stratified treatment allocation, including the use of additional pretransplant chemotherapy, under the assumption that a further reduction of tumor burden would optimize the benefit conferred by allogeneic HCT. However, so far, no well-controlled studies (eg, investigating immediate vs delayed transplantation in MRD-positive patients with available donors) have been conducted to rigorously test this hypothesis. Because MRD is fundamentally also an indicator for the reduced sensitivity of leukemia cells to prior therapies, the presence of residual disease could thus simply mark those patients who are unlikely to be cured with subsequent similar-type therapies, even if disease levels are brought temporarily below the level of detection. Moreover, additional therapy to reduce the tumor burden in patients with MRD is associated with a risk of complications, such as organ toxicities or infections, that could delay or prevent transplantation, increase transplant-related mortality, and offset any potential benefit of improved disease control.

Recommendation. There is currently no evidence to support or refute a benefit of additional chemotherapy for patients with intermediate-risk AML in first morphologic CR planned to undergo allogeneic HCT. The presence of MRD is not a contraindication to allogeneic HCT. Although MRD is associated with a several fold increased risk of post-HCT relapse even after adjustment for other predictive factors, up to 20% to 30% of patients with MRD at the time of transplantation experience prolonged DFS (ie, some MRD-positive patients will be salvaged with either myeloablative or nonmyeloablative conditioning allogeneic HCT).[5,31,32] Outside of a clinical trial, we suggest transplantation without additional chemotherapy in this situation. We acknowledge the controversy regarding the value of allogeneic HCT in first CR for intermediate-risk AML, for which chemotherapy-based postremission therapy followed by close observation and transplantation in second CR, if obtained, may be a reasonable alternative.[33]

Table 2. MRD detection in AML: remaining key issues

Detection method	Issue
All	Definition of MRD positivity/negativity
	Differences in source of material (bone marrow vs peripheral blood)
	For bone marrow: hemodilution
	Variation in timing/frequency of MRD sampling
	Regulatory approval/validation of assay
	Insufficient assay sensitivity
	Disease evolution with change in targets suitable for MRD detection
	Requirement of expertise for data interpretation
	Sample degradation
Molecular	Choice of target(s), target specificity
	Quality of cDNA synthesis
	Efficacy of PCR amplification
	Insufficient primer specificity
	Sensitivity of target gene overexpression limited by normal tissue expression
	Target stability
	Data normalization; choice of housekeeping gene
	Contamination
Flow cytometry	Choice of antigens and antibody panels
	Lack of immunophenotypic abnormalities
	Lack of diagnostic specimen to determine immunophenotypic abnormalities sufficient for MRD detection
	Choice of analysis strategy for MRD detection (diagnostic leukemia-associated immunophenotypes vs "different-from-normal" analysis)
	Lack of automatic analysis algorithms

cDNA, complementary DNA.

Key issues for MRD detection in AML have been previously highlighted by several investigators.[1-4,36,40,41,43,44,47-51]

Conclusion

In APL, molecular assessment of disease response has become standard practice, and MRD-directed therapy quite plausibly improves outcome, particularly in patients with high-risk disease.[34,35] In the other forms of AML, attempts to measure MRD are complicated by the genetic and molecular complexity/diversity at initial presentation and disease evolution over the course of the illness with the possibility that minor subclones can emerge at the time of recurrence.[36,37] Not all abnormalities are therefore equally suited as MRD parameters. Nonetheless, MRD is now established as an independent marker of increased relapse risk in non-APL AML and may be able to replace morphologic examinations as the gold standard for the assessment of treatment responses.[4] On the other hand, conclusive data on the value of MRD-based, risk-stratified therapy is currently not available. A widely cited study in pediatric AML has used a combination of MRD measurement and genetic disease features to direct decisions on the second induction course and subsequent therapy, and based on a comparison with previous treatment cohorts suggested that this approach could improve

outcomes.[38] However, because of patient heterogeneity and improving supportive care measures, comparisons with "historic" control groups can be problematic,[39] and better controlled and ideally randomized studies will ultimately be required to make a compelling argument for MRD-directed interventions in non-APL AML.

APL exemplifies that periodic MRD monitoring for patients with acute leukemia can be adopted as "standard" once its value is demonstrated. The impact of MRD monitoring in non-APL AML on survival, quality of life, and resource utilization is currently being explored in the United Kingdom's NCRI AML17 trial. In this trial, patients with leukemias that have informative molecular markers are randomized to a "MRD monitoring vs no MRD monitoring" strategy, with the question of therapeutic intervention being left to the primary hematologist/oncologist. So far, however, the use of MRD monitoring as a routine tool in non-APL AML is hampered by interlaboratory differences in the assays and preferred analytical methods, varying approaches to defining MRD positivity/negativity with need to identify the cutoff values that are most informative at a given time point, differences in source of material (bone marrow vs peripheral blood) and correction for hemodilution if marrow is examined, and variation in the exact timing and frequency of MRD sampling. The remaining key issues with respect to MRD detection in AML are summarized in Table 2. Inconsistencies in MRD assays limit and complicate their interpretation and transferability of results and, likely, curb the enthusiasm of regulatory authorities to use MRD as an end point in the drug approval process. As an important step toward optimized use of MRD assays, it will be critical to address these current shortcomings through the adoption of standardized methodologic approaches with frequent external quality control, and validation and clarification of regulatory considerations. Although their need is well recognized, efforts in that direction have only begun.[4,40-44] In light of these limitations, we would highly encourage the research community to work toward standardized methods for the detection and monitoring of MRD levels and use them as soon as they become available, and to conduct well controlled, ideally randomized trials for evaluating the value of MRD-directed treatment escalation or de-escalation in AML. Recent studies in acute lymphoblastic leukemia demonstrate that such trials are feasible and can provide definitive evidence that modification of postremission treatment intensity based on MRD status can optimize treatment outcomes.[45,46]

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Authorship

Contribution: S.K. and R.B.W. were responsible for the concept of this review, contributed to the literature search data collection/quality assessment, analyzed and interpreted data, and wrote the manuscript; R.F.S. and D.G. analyzed and interpreted data, and critically revised the manuscript; and V.E.D.Y. designed the literature search, performed the data extraction and quality assessment, analyzed and interpreted data, and critically revised the manuscript.

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