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MINIMAL RESIDUAL DISEASE QUANTITATION IN ACUTE MYELOID LEUKEMIA

David Shook, M.D.[1], **Elaine Coustan-Smith, M.S.**[1], **Raul C. Ribeiro, M.D.**[1,3], **Jeffrey E. Rubnitz, M.D., Ph.D.**[1,3], and **Dario Campana, M.D., Ph.D.**[1,2,3]

[1]Department of Oncology, St. Jude Children's Research Hospital

[2]Department of Pathology, St. Jude Children's Research Hospital

[3]Department of Pediatrics, University of Tennessee Health Science Center, Memphis, TN

Abstract

The prognosis for patients with acute myeloid leukemia (AML) is heterogeneous. A minority of patients has clinical and biologic features that are associated with a very high risk of relapse. For the remaining patients no clear prognostic factors can be identified at diagnosis. The degree of treatment response is likely to be an informative predictor of outcome for these patients. Modern assays to detect AML cells that are undetectable by conventional morphologic techniques, i.e. minimal residual disease (MRD), can potentially improve measurements of treatment response. It is plausible that modifications to treatment based on the results of these assays will improve clinical management and ultimately increase cure rates. Established MRD assays for AML are based on either polymerase chain reaction (PCR) amplification of genetic abnormalities or flow cytometric detection of abnormal immunophenotypes. Residual disease and treatment response can be measured by these assays in a manner that is much more sensitive and objective than that afforded by conventional morphologic examination. The expanding use of MRD testing is beginning to change the definition of treatment response and of remission. Other clinically informative uses of MRD testing include the detection of early relapse and the evaluation of the efficacy of new antileukemic agents.

Keywords

acute myeloid leukemia; minimal residual disease; flow cytometry; PCR

Introduction

Survival among patients with acute myeloid leukemia (AML) has failed to increase commensurately with our improved understanding of the disease biology. While the risk of relapse can be predicted with some accuracy in a minority of patients, based on presenting clinical and/or biologic features, risk assignment remains difficult for the majority of patients.[1,2] Because of the increasing availability of novel agents and improvements in supportive therapy, it has become more urgent to identify patients who require more intensive therapy and experimental treatment, and those who have low-risk disease that is curable with conventional therapies. Patients who lack well-defined prognostic features at diagnosis (“standard risk”)

Corresponding Author: Dario Campana, M.D., Ph.D., Department of Oncology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis TN 38105-3678; Telephone 901-595 2528; FAX 901-595 5947; dario.campana@stjude.org.

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compose the majority of patients with AML; this subgroup has the greatest heterogeneity in response and potentially has the most to gain from improvements in definition of risk hazard.

Although still integral in following the effects of therapy on normal and abnormal cells, traditional morphologic examination of blood and marrow has limited sensitivity and specificity as a method for response evaluation. Therefore, by this approach, there is a risk of either over- or under-estimating disease burden. During the last 3 decades efforts to develop ways to measure the degree of residual leukemia better than morphology and to detect minimal residual disease (MRD) have progressively intensified resulting in clinically applicable methods. Efforts in the area of MRD testing for acute lymphoblastic leukemia and acute promyelocytic leukemia (APL) led the way.[3-6] More recently, however, reliable methods to detect MRD in non-APL AML have been established and their clinical importance has been demonstrated. MRD testing replaces the inherent limitations of morphologic evaluation, with a one hundred-fold improved sensitivity and objective identification of leukemia-associated features. We will briefly review the clinical significance of MRD in AML, advantages and limitation of the available methods and future uses of MRD in clinical practice. The clinical utility of MRD monitoring in APL will not be discussed here.

Clinical significance of MRD

The potential clinical utility of monitoring MRD in AML has been demonstrated by several studies.[4,5,7] Table 1 summarizes key findings in selected studies with the two methodologies that are most widely used: polymerase chain reaction (PCR) amplification of molecular abnormalities, and flow cytometric detection of abnormal immunophenotypes.

Quantitative PCR studies for molecular detection of AML1-ETO and CBFbeta/MYH11 in cases with t(8;21) and inv(16), respectively, have shown that these assays can identify patients at a higher risk of relapse.[8-16]

While studies of MRD by PCR amplification of fusion transcripts are inherently limited to specific leukemic subtypes, studies of MRD by flow cytometry have a potentially much wider applicability. San Miguel et al.[17] showed that in adult patients with AML in morphologic remission, MRD detected in the first bone marrow obtained after induction treatment was highly predictive or relapse. By MRD levels, patients could be subdivided into 4 groups: none of the 8 with fewer than 0.01% leukemic cells had relapsed at the time of publication, while the relapse rate for the 17 with more than 1% residual cells was 84%; patients with 0.01%-0.1% leukemic cells (n = 37) and those with 0.1%-1% (n = 64) had an intermediate relapse rate. Kern et al.[18] monitored MRD in AML patients in complete remission after induction and consolidation therapy and found that the degree of reduction in cells expressing aberrant immunophenotypes was significantly and independently related to treatment outcome. Early studies by Venditti and colleagues also showed the clinical importance of MRD in adult patients with AML.[19] More recently, this group reported a studied that provided strong evidence supporting the clinical importance of this parameter.[20] These authors studied 142 patients who achieved complete remission and found that a level of 0.035% MRD after consolidation therapy was very informative, identifying two groups of patients with a 5-year relapse-free survival of 60% and 16%, respectively. MRD-negative patients had a significantly more favorable outcome, regardless of whether they received autologous or allogeneic stem cell transplantation. A recent study by Al Mawali et al.[21] suggested that the most informative MRD level after induction therapy was 0.15%, although this series included only 25 patients.

The Children's Oncology Group completed a prospective study of MRD in 252 children with AML. Occult leukemia was detected by flow cytometry in 41 (16%) of the patients who responded to initial induction therapy.[22] While controlling for other disease and patient characteristics, patients with detectable disease were nearly five times more likely to relapse.

In a study performed with samples collected from children enrolled in the St. Jude Children's Research Hospital AML97 study, we found that 61 of 230 bone marrow samples from 46 patients during therapy had MRD levels of 0.1% or higher.[23] Mean (± SE) 2-year overall survival was 33.1% ± 19.1% for patients with detectable MRD (≥ 0.1%) after induction therapy as compared to 72.1% ± 11.5% for those with undetectable MRD; AML relapse within the subsequent 6 months was significantly more likely in the MRD-positive group. MRD was the only significant prognostic indicator in this cohort. In a study by Langebrake et al.[24] in 150 children with AML residual disease detected by flow cytometry was significantly associated with a lower event-free survival. However, when the prognostic value of the findings was assessed in combination with that of other parameters, residual disease lost statistical significance.

Methods for MRD monitoring in AML

Polymerase chain reaction

The fusion transcripts most extensively used to monitor MRD in AML (in addition to PML-RARA for APL) are AML1-ETO, CBFbeta-MYH11 and MLL-AF9 which are present in approximately one-third of non-APL AML cases.[5] The detection of abnormal fusion transcripts by qualitative reverse transcriptase (RT)-PCR may persist long into clinical remission. Thus, the use of quantitative methods to estimate levels of target genes and by inference, levels of MRD at different phases of the disease, are preferred.[9] The current standard method for molecular quantitative detection of MRD is real-time quantitative PCR (RQ-PCR).[5,25-27] RQ-PCR rapidly quantifies PCR products by real-time fluorescent signals during exponential amplification.[28] Moreover, it does so without additional post-PCR processing and associated risk of contamination.

The sensitivity of molecular detection of fusion transcripts ranges from 1 leukemic cell in 1,000 to 100,000 normal cells, i.e. 0.1% to 0.001%. Although RQ-PCR can precisely quantify PCR products, the relation between these and the number of cells from which they originate might be difficult to extrapolate. The transcript : cell ratio may vary among leukemias of the same genetic subtype,[25,29] and perhaps also between cells collected at diagnosis and during therapy, and between cells at different maturation stages within the leukemic clone. Moreover, RNA is prone to degradation and the efficiency of its initial conversion to cDNA by reverse transcriptase can fluctuate. In sum, although very useful to determine presence or absence of MRD, this method has some limitations in regards to the precise assessment of MRD levels.

In addition to fusion transcripts, there are other potential targets for molecular studies of MRD in AML. The Wilms' tumor gene (*WT1*) is highly expressed in most acute leukemias, and its detection in bone marrow has been associated with the presence, persistence, or reappearance of leukemia,[30-33] but the background of normal bone marrow cells may limit the reliability of this assay in many cases. In some cases of AML, cells have an internal tandem duplication of the *FLT3* gene (*FLT3*/ITD),[34] which is detected in approximately 25% of adult AML cases, [35] and 15% childhood AML.[36,37] Although methods for detecting *FLT3*/ITD for MRD monitoring purposes have been developed,[38] the observation that this marker may not be stable during the course of the disease[39,40] argues against its use as MRD target. The value of MRD studies using nucleophosmin mutations as targets is beginning to be explored but definitive data are not yet available.[41,42] Immunoglobulin and T-cell receptor genes, extensively used as targets for molecular analysis of MRD in acute lymphoblastic leukemia, are rarely rearranged in AML and therefore their contribution to the arsenal of potential targets is negligible.[43]

Flow Cytometry

Leukemic cells express abnormal patterns of cellular markers, and these aberrant immunophenotypes can be identified by flow cytometry. AML cells express unique cell marker and light scatter profiles that allow their identification among normal bone marrow myeloid cells.[4445] This phenotypic signature can include not only differential expression of myeloid markers but also expression of non-myeloid markers as well as a mixture of markers representing normally distinct maturation stages.[46]

Published adult AML studies report identifying abnormal phenotypes in approximately 70%-75% of patients.[17,19] In an early study, we found abnormal immunophenotypes in 46 of 54 (85.2%) children with newly diagnosed AML.[23] In about half of the cases the immunophenotypes were sufficiently distinct from those of normal bone marrow cells to allow a sensitivity of detection of 1 leukemic cell among 10,000 normal cells. In the remaining cases, the immunophenotypes were aberrant but overlapped in part with those of normal myeloid cells, limiting the sensitivity of the assay to 1 in 1000. In the studies performed in our recently closed AML02 trial, we found aberrant immunophenotypes at diagnosis in 200 of 210 (95%) patients. As we previously noted, in approximately half of the patients, the antigenic expression was sufficiently distinct from that of normal cells to allow a sensitivity of 1 in 10,000. However, because this is not achievable in all patients, our standard definition of MRD positivity in AML is $\geq 0.1\%$.

An advantage of flow cytometry-based studies of MRD is that they can accurately quantify residual leukemic cells. Moreover, they can distinguish viable from apoptotic cells and provide an overview of the status of normal hematopoietic cells in the sample. The issue of immunophenotypic changes that may occur during the course of the disease is often brought up as a potential cause of false-negative results. Indeed, immunophenotypic switches of markers or changes in signal intensity may occur but, in general, some or all the phenotypic abnormalities described at diagnosis reappear at the time of relapse.[47-49] We strongly recommend the use of multiple antibody sets to maximize the chances of capturing the residual AML cells even when partial changes in phenotype have occurred and thus reduce the likelihood of false-negative findings. A second potential cause of erroneous findings is related to the fact that current flow cytometry-based MRD assays rely on the interpretative abilities of the investigator. Lack of extensive knowledge about the immunophenotypic features of normal bone marrow cells, and of the changes that may occur during bone marrow recovery after chemotherapy may lead to erroneous interpretation. Thus, an excess of highly proliferating immature myeloid cells may be mistakenly identified as residual myeloid cells. Therefore, in addition to specific training, it is important to use rigorous gating strategies based on objective findings in an extensive database of normal and regenerating bone marrow samples. In this regard, the development of software that allows automated analysis of flow cytometric data might be very helpful.[50]

Future prospects

AML is a heterogeneous malignancy with a great need for improved therapeutic strategies. MRD assays are capable of producing information on treatment response with more sensitivity than microscopic analysis. Thus, MRD can be used to design risk-based therapies for AML in an effort to minimize toxicity and improve cure rates.[7] The strategy that we used in our AML02 trial is shown in Figure 1. Quantitative RT-PCR and flow cytometry are currently the two most effective methods for MRD measurement. Because these are high-complexity tests, they need to be performed in laboratories with proven expertise to be beneficial.

There are other applications of MRD assays that are beginning to be implemented. MRD studies can be used to assess the effectiveness of novel antileukemic agents. Thus, changes in MRD

levels could represent an endpoint in phase II studies. A related application of MRD is to rapidly determine the effectiveness of remission induction chemotherapy regimens. In other words, prevalence and levels of MRD after a chemotherapy phase can be compared to data obtained in previous or parallel trials to decide whether the tested regimen is superior or not. In this context, MRD data can be part of stopping rules.

An increasing number of tyrosine kinase inhibitors are becoming available for clinical testing in AML. An exciting new possibility is to assess the status of cell signaling pathways targeted by these inhibitors in AML cells. These studies have become possible owing to the increasing battery of antibodies directed against phosphoproteins that can be visualized by flow cytometers designed to detect 9 or more fluorochromes.[51] By using these techniques, one can determine if the targeted signaling pathway is affected in the residual leukemic cells.

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REFERENCES

1. Stone RM, O'Donnell MR, Sekeres MA. Acute myeloid leukemia. Hematology Am Soc Hematol Educ Program 2004:98–117. [PubMed: 15561679]
2. Ravandi F, Burnett AK, Agura ED, et al. Progress in the treatment of acute myeloid leukemia. Cancer 2007;110:1900–10. [PubMed: 17786921]
3. Szczepanski T, Orfao A, van der Velden VH, et al. Minimal residual disease in leukaemia patients. Lancet Oncology 2001;2:409–17. [PubMed: 11905735]
4. Campana D. Determination of minimal residual disease in leukemia patients. Br J Haematol 2003;121:823–38. [PubMed: 12786792]
5. Liu YJ, Grimwade G. Minimal residual disease evaluation in acute myeloid leukaemia. Lancet 2002;360:160–2. [PubMed: 12126839]
6. Lo-Coco F, Ammatuna E. The biology of acute promyelocytic leukemia and its impact on diagnosis and treatment. Hematology Am Soc Hematol Educ Program 2006:156–61, 514. [PubMed: 17124055]
7. Goulden N, Virgo P, Grimwade G. Minimal residual disease directed therapy for childhood acute myeloid leukaemia: the time is now. Br.J.Haematol 2006;134:273–82. [PubMed: 16848770]
8. Marcucci G, Livak KJ, Bi W, et al. Detection of minimal residual disease in patients with AML1/ETO-associated acute myeloid leukemia using a novel quantitative reverse transcription polymerase chain reaction assay. Leukemia 1998;12:1482–9. [PubMed: 9737700]
9. Tobal K, Newton J, Macheta M, et al. Molecular quantitation of minimal residual disease in acute myeloid leukemia with t(8;21) can identify patients in durable remission and predict clinical relapse. Blood 2000;95:815–9. [PubMed: 10648391]
10. Guerrasio A, Pilatrino C, De Micheli D, et al. Assessment of minimal residual disease (MRD) in CBFbeta/MYH11-positive acute myeloid leukemias by qualitative and quantitative RT-PCR amplification of fusion transcripts. Leukemia 2002;16:1176–8. [PubMed: 12040450]
11. Viehmann S, Teigler-Schlegel A, Bruch J, et al. Monitoring of minimal residual disease (MRD) by real-time quantitative reverse transcription PCR (RQ-RT-PCR) in childhood acute myeloid leukemia with AML1/ETO rearrangement. Leukemia 2003;17:1130–6. [PubMed: 12764380]
12. Marcucci G, Caligiuri MA, Dohner H, et al. Quantification of CBFbeta/MYH11 fusion transcript by real time RT-PCR in patients with INV(16) acute myeloid leukemia. Leukemia 2001;15:1072–80. [PubMed: 11455976]
13. Buonamici S, Ottaviani E, Testoni N, et al. Real-time quantitation of minimal residual disease in inv (16)-positive acute myeloid leukemia may indicate risk for clinical relapse and may identify patients in a curable state. Blood 2002;99:443–9. [PubMed: 11781223]

14. van der Reijden BA, Simons A, Luiten E, et al. Minimal residual disease quantification in patients with acute myeloid leukaemia and inv(16)/CBFB-MYH11 gene fusion. Br J Haematol 2002;118:411–8. [PubMed: 12139724]
15. Leroy H, de Botton S, Grardel-Duflos N, et al. Prognostic value of real-time quantitative PCR (RQ-PCR) in AML with t(8;21). Leukemia 2005;19:367–72. [PubMed: 15674426]
16. Perea G, Lasa A, Aventin A, et al. Prognostic value of minimal residual disease (MRD) in acute myeloid leukemia (AML) with favorable cytogenetics [t(8;21) and inv(16)]. Leukemia 2006;20:87–94. [PubMed: 16281071]
17. San Miguel JF, Vidriales MB, Lopez-Berges C, et al. Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to postinduction treatment stratification. Blood 2001;98:1746–51. [PubMed: 11535507]
18. Kern W, Voskova D, Schoch C, et al. Determination of relapse risk based on assessment of minimal residual disease during complete remission by multiparameter flow cytometry in unselected patients with acute myeloid leukemia. Blood 2004;104:3078–85. [PubMed: 15284114]
19. Venditti A, Buccisano F, Del Poeta G, et al. Level of minimal residual disease after consolidation therapy predicts outcome in acute myeloid leukemia. Blood 2000;96:3948–52. [PubMed: 11090082]
20. Maurillo L, Buccisano F, Del Principe MI, et al. Toward optimization of postremission therapy for residual disease-positive patients with acute myeloid leukemia. J Clin.Oncol 2008;26:4944–51. [PubMed: 18606980]
21. Al-Mawali A, Gillis D, Lewis I. The use of receiver operating characteristic analysis for detection of minimal residual disease using five-color multiparameter flow cytometry in acute myeloid leukemia identifies patients with high risk of relapse. Cytometry B 2009;76B:91–101.
22. Sievers EL, Lange BJ, Alonzo TA, et al. Immunophenotypic evidence of leukemia after induction therapy predicts relapse: results from a prospective Children's Cancer Group study of 252 acute myeloid leukemia patients. Blood 2003;101:3398–406. [PubMed: 12506020]
23. Coustan-Smith E, Ribeiro RC, Rubnitz JE, et al. Clinical significance of residual disease during treatment in childhood acute myeloid leukemia. Br J Haematol 2003;123:243–52. [PubMed: 14531905]
24. Langebrake C, Creutzig U, Dworzak M, et al. Residual disease monitoring in childhood acute myeloid leukemia by multiparameter flow cytometry: the MRD-AML-BFM Study Group. J Clin Oncol 2006;24:3686–92. [PubMed: 16877738]
25. Gabert J, Beillard E, van der Velden V, et al. Standardization and quality control studies of ‘real-time’ quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. Leukemia 2003;17:2318–57. [PubMed: 14562125]
26. van der Velden V, Hochhaus A, Cazzaniga G, et al. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia 2003;17:1013–34. [PubMed: 12764363]
27. Freeman SD, Jovanovic JV, Grimwade G. Development of minimal residual disease-directed therapy in acute myeloid leukemia. Semin Oncol 2008;35:388–400. [PubMed: 18692689]
28. van der Velden V, Hochhaus A, Cazzaniga G, et al. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia 2003;17:1013–34. [PubMed: 12764363]
29. Lion T. Current recommendations for positive controls in RT-PCR assays. Leukemia 2001;15:1033–7. [PubMed: 11455970]
30. Bergmann L, Miething C, Maurer U, et al. High levels of Wilms' tumor gene (wt1) mRNA in acute myeloid leukemias are associated with a worse long-term outcome. Blood 1997;90:1217–25. [PubMed: 9242555]
31. Cilloni D, Gottardi E, Fava M, et al. Usefulness of quantitative assessment of the WT1 gene transcript as a marker for minimal residual disease detection. Blood 2003;102:773–4. [PubMed: 12835231]
32. Weisser M, Kern W, Rauhut S, et al. Prognostic impact of RT-PCR-based quantification of WT1 gene expression during MRD monitoring of acute myeloid leukemia. Leukemia 2005;19:1416–23. [PubMed: 15920493]

33. Lapillonne H, Renneville A, Auvrignon A, Flamant C, Blaise A, Perot C, et al. High WT1 expression after induction therapy predicts high risk of relapse and death in pediatric acute myeloid leukemia. J Clin Oncol 2006;24:1507–15. [PubMed: 16575000]
34. Nakao M, Janssen JW, Erz D, et al. Tandem duplication of the FLT3 gene in acute lymphoblastic leukemia: a marker for the monitoring of minimal residual disease. Leukemia 2000;14:522–4. [PubMed: 10720156]
35. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. Blood 2001;98:1752–9. [PubMed: 11535508]
36. Meshinchi S, Woods WG, Stirewalt DL, et al. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. Blood 2001;97:89–94. [PubMed: 11133746]
37. Meshinchi S, Stirewalt DL, Alonzo TA, et al. Structural and numerical variation of FLT3/ITD in pediatric AML. Blood 2008;111:4930–3. [PubMed: 18305215]
38. Beretta C, Gaipa G, Rossi V, et al. Development of a quantitative-PCR method for specific FLT3/ITD monitoring in acute myeloid leukemia. Leukemia 2004;18:1441–4. [PubMed: 15201851]
39. Kottaridis PD, Gale RE, Langabeer SE, et al. Studies of FLT3 mutations in paired presentation and relapse samples from patients with acute myeloid leukemia: implications for the role of FLT3 mutations in leukemogenesis, minimal residual disease detection, and possible therapy with FLT3 inhibitors. Blood 2002;100:2393–8. [PubMed: 12239147]
40. Shih LY, Huang CF, Wu JH, et al. Internal tandem duplication of FLT3 in relapsed acute myeloid leukemia: a comparative analysis of bone marrow samples from 108 adult patients at diagnosis and relapse. Blood 2002;100:2387–92. [PubMed: 12239146]
41. Barragan E, Pajuelo JC, Ballester S, et al. Minimal residual disease detection in acute myeloid leukemia by mutant nucleophosmin (NPM1): comparison with WT1 gene expression. Clin Chim Acta 2008;395:120–3. [PubMed: 18590714]
42. Gorello P, Cazzaniga G, Alberti F, et al. Quantitative assessment of minimal residual disease in acute myeloid leukemia carrying nucleophosmin (NPM1) gene mutations. Leukemia 2006;20:1103–8. [PubMed: 16541144]
43. Boeckx N, Willemse MJ, Szczepanski T, et al. Fusion gene transcripts and Ig/TCR gene rearrangements are complementary but infrequent targets for PCR-based detection of minimal residual disease in acute myeloid leukemia. Leukemia 2002;16:368–75. [PubMed: 11896540]
44. Terstappen LW, Loken MR. Myeloid cell differentiation in normal bone marrow and acute myeloid leukemia assessed by multi-dimensional flow cytometry. Anal Cell Pathol 1990;2:229–40. [PubMed: 1703434]
45. Campana D, Coustan-Smith E, Janossy J. The immunologic detection of minimal residual disease in acute leukemia. Blood 1990;76:163–71. [PubMed: 1973061]
46. San Miguel JF, Martinez A, Macedo A, et al. Immunophenotyping investigation of minimal residual disease is a useful approach for predicting relapse in acute myeloid leukemia patients. Blood 1997;90:2465–70. [PubMed: 9310499]
47. Macedo A, San Miguel JF, Vidriales MB, et al. Phenotypic changes in acute myeloid leukaemia: implications in the detection of minimal residual disease. J Clin Pathol 1996;49:15–8. [PubMed: 8666678]
48. Baer MR, Stewart CC, Dodge RK, et al. High frequency of immunophenotype changes in acute myeloid leukemia at relapse: implications for residual disease detection (Cancer and Leukemia Group B Study 8361). Blood 2001;97:3574–80. [PubMed: 11369653]
49. Kern W, Haferlach C, Haferlach T, et al. Monitoring of minimal residual disease in acute myeloid leukemia. Cancer 2008;112:4–16. [PubMed: 18000811]
50. Pedreira CE, Costa ES, Almeida J, et al. A probabilistic approach for the evaluation of minimal residual disease by multiparameter flow cytometry in leukemic B-cell chronic lymphoproliferative disorders. Cytometry A 2008;73A:1141–1150. [PubMed: 18836994]
51. Irish JM, Kotecha N, Nolan GP. Mapping normal and cancer cell signaling networks: towards single-cell proteomics. Nat Rev Cancer 2006;6:146–55. [PubMed: 16491074]

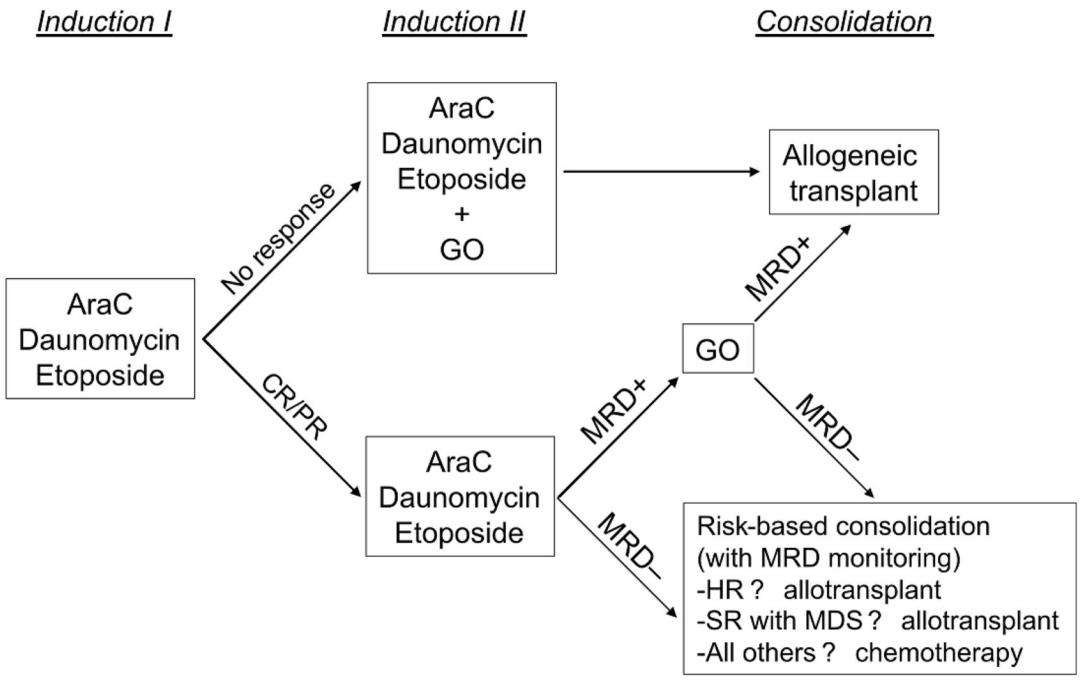

FIGURE 1.

APPLICATION OF MRD TESTING FOR RISK STRATIFICATION IN THE ST JUDE AML02 STUDY FOR CHILDREN WITH AML (APL EXCLUDED). Abbreviations: GO, gemtuzumab ozogamicin; CR/PR, complete response, partial response; HR, high risk; SR, standard risk; MDS, myelodysplastic syndrome.

TABLE 1

RESULTS OF SELECTED MRD STUDIES IN AML

Subtype	Target gene/ control gene	Patient number	Informative timepoint	Method	Informative MRD level	Reference
Adults: t(8;21)	*AML1-ETO/ TBP* ratio	21	Post-consolidation	RQ-PCR	Transcript: control >10^{-5}	Leroy et al, 2005[15]
All adults	*WT1/ABL*	116	61-120 days & 121-180 days post start of chemotherapy	RQ-PCR	>0.4 %	Weisser et al, 2005[32]
Adults: inv(16)	*CBFB-MYH11/ABL*	21	Post-completion of chemotherapy	RQ-PCR	>0.25 %	Buonamici et al, 2002[13]
Adults: t(8;21)	*AML1-ETO/ABL*	25	Post-consolidation / intensification	RT-PCR	1000 mol/mg of RNA	Tobal et al, 2000[9]
Adults: t(8;21) inv(16)	*AML1-ETO/ABL*	28	Post-completion of chemotherapy	RQ-PCR	>10 transcript copies	Perea et al, 2006[16]
	CBFB-MYH11/ABL	27		FC	>0.1 %	
All adults	Aberrant phenotypes	126	Post-first induction	FC	>0.01 %	San Miguel et al, 2001[17]
All adults	Aberrant phenotypes	142	Post-remission	FC	≥0.035 %	Maurillo et al, 2008[20]
All children	Aberrant phenotypes	252	Post-first induction	FC	≥0.5%	Sievers et al, 2003[22]
All children	Aberrant phenotypes	46	Post-first induction	FC	≥0.1%	Coustan-Smith et al, 2003[23]
All children	Aberrant phenotypes	150	Prior to second induction	FC	>0.1%	Langebrake et al, 2006[24]

Abbreviations: RQ-PCR, real-time polymerase chain reaction; FC, flow cytometry