Evidence-Based Focused Review

blood

Minimal residual disease–guided therapy in childhood acute lymphoblastic leukemia

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Case presentations

Case 1

A 3-year-old boy presented with a leukocyte count of 5.9×10^9/L and was found to have near-haploid B-lineage acute lymphoblastic leukemia (ALL) with a 27, X, +Y, +13, +18, +21 karyotype. He was enrolled in the St. Jude Children's Research Hospital Total Therapy XV study. After 19 days of remission induction therapy with 1 high dose of methotrexate, 14 days of prednisone, 2 doses of vincristine and daunorubicin, and 6 doses of *Escherichia coli*-derived asparaginase, flow cytometry examination of his bone marrow revealed the presence of minimal residual disease (MRD) amounting to 3 leukemic cells per 10 000 mononucleated cells (0.03%). Upon completion of the remaining remission induction therapy consisting of 1 dose of cyclophosphamide, 14 days of mercaptopurine, and 8 once-per-day doses of cytarabine, he attained a morphologic remission on day 46, with undetectable (<0.01%) MRD by flow cytometry and polymerase chain reaction. Because of the near-haploid ALL karyotype and negative day 46 MRD, he was assigned to receive intensive chemotherapy for 3 years. MRD remained undetectable throughout treatment. He has remained in continuous complete remission for 11.6 years.

Case 2

A 9-year-old boy presented with a 3-month history of progressive pallor and upper respiratory tract infection. He had no hepatosplenomegaly or mediastinal mass. An abnormal blood count with hemoglobin 3.4 g/dL, leukocytes 4.2×10^9/L with 15% neutrophils, 52% lymphocytes, 33% blasts, and platelets 123×10^9/L prompted a bone marrow examination which disclosed 96% replacement with leukemic lymphoblasts. By flow cytometry, the blasts expressed CD45, surface CD3, CD2, CD7, T-cell receptor γ/δ, CD11b, and CD13, with a subset of cells positive for CD56, a cell profile indicative of T-cell ALL. He was enrolled on the Total Therapy XV study and began remission induction therapy with high-dose methotrexate followed by prednisone once per day, vincristine once per week, daunorubicin once per week, and *E coli*–derived asparaginase 3 times per week. On day19 of treatment, 62.9% of bone marrow mononucleated cells were leukemic lymphoblasts by flow cytometry (31% of all cells were blasts by morphology). Three additional doses of asparaginase were given, and the remaining remission induction therapy consisted of cyclophosphamide, mercaptopurine, and cytarabine. On day 46, he attained morphologic remission (with 3% lymphoblasts), but MRD by flow cytometry was 5.82%. After consolidation treatment with 4 courses of high-dose methotrexate plus mercaptopurine as well as 1 course of re-intensification therapy with dexamethasone, etoposide, high-dose cytarabine, and asparaginase, MRD decreased to 0.18%. He attained MRD-negative status (<0.01%) after a second course of re-intensification treatment and subsequently underwent a matched-related allogeneic hematopoietic stem cell transplantation. He has remained in continuous complete remission for 11.9 years.

Introduction

The first report of minimal (ie, not morphologically evident) residual disease (MRD) in leukemia was published nearly 4 decades ago.[1] By identifying leukemic cells with fluorochrome-conjugated antisera and fluorescence microscopy, this study disclosed their presence in the bone marrow of patients with ALL after remission induction therapy. Thus, a fundamental concept in the modern evaluation and management of acute leukemia was introduced: bone marrow in complete remission may contain leukemic cells detectable by methods that are more sensitive and objective than morphologic examination.

The initial microscopic methods were subsequently replaced by flow cytometry, and the number and quality of antibodies available for leukemia immunophenotyping progressively increased.[2] The expanding knowledge about the marker profile of leukemic cells together with improvements in technology led to flow cytometric methods that can identify a distinctive leukemia-associated immunophenotype in virtually all patients with ALL and can reliably detect 1 leukemic cell among 10 000 or more normal bone marrow or peripheral blood cells.[3-6] In parallel, an impressive development has occurred for molecular methods to detect MRD in ALL, which target clonal rearrangements of immunoglobulin and/or T-cell receptor genes. By amplifying these unique molecular signatures using patient-specific polymerase chain reaction (PCR) primers or, as shown more recently, by subjecting PCR-amplified DNA fragments of these genes to deep-sequencing analysis, MRD at levels of 1 in 100 000 or more cells can be detected.[6,7] The application of flow cytometry and PCR to monitor MRD in patients with ALL consolidated the notion that residual leukemia can be present at various levels during treatment among patients who are in clinical complete remission without morphologically evident disease.[5,6] Some patients achieve MRD-negative status, typically defined as <0.01% leukemic cells in bone marrow and peripheral blood after remission induction therapy. Other patients harbor MRD at levels that can range from 0.01% to 5% or more.

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Table 1. MRD stratification for patients with B-lineage ALL in selected contemporary clinical trials

Study	MRD study		MRD stratification			
	Time	Site				
Associazione Italiana Ematologia Oncologia Pediatrica Berlin-Frankfurt-Münster (AIEOP-BFM) ALL 2009[8,9]*	Day 15 Day 33 Day 78	Bone marrow Bone marrow Bone marrow	SR No HR factors and day 33 and day 78 MRD negative by PCR, or PCR-MRD not available and day 15 <0.1% by FCM	MR Others	HR HR factors, or day 15 ≥10% by FCM or day 78 ≥0.05% by PCR, or for B-ALL only, day 33 ≥0.05% and day 78 positive <0.05% by PCR	
Children's Oncology Group (COG) AALL 08B1[10]†	Day 8 Day 29	Blood Bone marrow	LR NCI SR Favorable genetics; no unfavorable factors; day 8 <0.01%; day 29 <0.01%	AR NCI SR Favorable genetics; no unfavorable factors; day 8 ≥0.01%; day 29 <0.01%, or neutral genetics; no unfavorable factors; day 8 <1%; day 29 <0.01%	HR NCI SR Favorable genetics; no unfavorable factors; day 29 ≥0.01% NCI SR Neutral genetics; no unfavorable factors; day 8 ≥1%; day 29 <0.01% NCI HR Day 29 <0.01%	VHR NCI SR or HR Unfavorable factors NCI SR Neutral genetics; day 29 ≥0.01% NCI HR Day 29 ≥0.01%, or Age ≥13 y
Dutch Children's Oncology Group (DCOG) ALL-10[12]‡	Day 33 Day 79	Bone marrow Bone marrow	SR No unfavorable factors; day 33 and day 79 undetectable by PCR	MR Others	HR Unfavorable factors; day 33 ≥0.05%; day 79 ≥0.05%	Induction failure Day 29 >5%
NCRI United Kingdom Acute Lymphoblastic Leukaemia (UKALL) 2011[13,14]§	Day 29 Week 14	Bone marrow Bone marrow	LR Day 29 <0.005%	IR Unfavorable factors, or Day 29 <0.005%; and week 14 <0.5%	HR Week 14 ≥0.5%	
St. Jude Children's Research Hospital (SJCRH) Total Therapy XVI[17-19]‖	Day 15 Day 42 Week 15	Bone marrow Bone marrow Bone marrow if day 42 ≥0.01%	LR Favorable factors; day 15 <1%; day 42 <0.01%	SR Favorable factors; day 15 ≥1%; day 42 <1%, or Unfavorable factors; day 42 <1%	HR Day 42 ≥1%, or week 15 ≥0.1%	

AIEOP, Associazione Italiana Ematologia Oncologia Pediatrica; AR, average risk; FM, Berlin-Frankfurt-Münster; COG, Children's Oncology Group; DCOG, Dutch Children's Oncology Group; FCM, flow cytometry; HR, high risk; IR, intermediate risk; LR, low risk; MR, medium risk; NCI, National Cancer Institute; SJCRH, St. Jude Children's Research Hospital; SR, standard risk; UKALL, United Kingdom Acute Lymphoblastic Leukaemia; VHR, very high risk.

*High-risk factors include prednisone poor response, nonremission on day 33, *MLL-AF4* or t (4;11), hypodiploidy <45 chromosomes.

†Favorable genetics include double trisomy 4 and 10 or *ETV6-RUNX1*, and unfavorable factors include central nervous system 3 (CNS3) status, testicular leukemia, hypodiploidy <44 chromosomes, DNA index <0.81, intrachromosomal amplification of chromosome 21, M3 marrow on day 29, *MLL* rearrangement, and *BCR-ABL1*. NCI HR indicates patients with B-lineage ALL with presenting leukocyte count ≥50 000/μL or age ≥10 y, and NCI SR indicates patients without these features.

‡Unfavorable factors include *MLL-AF4*, prednisone poor response, CNS3 status, testicular leukemia, and no complete remission on day 33.

§Unfavorable factors include *MLL* rearrangement, near haploidy <30 chromosomes, low hypoploidy 30-39 chromosomes, t(17;19) (q23;p13) and intrachromosomal amplification of chromosome 21.

‖Favorable factors include NCI SR, *ETV6-RUNX1*, DNA index ≥1.16; unfavorable factors include CNS3 status, testicular leukemia, *BCR-ABL1*, *E2A-PBX1*, *MLL* rearrangement, and hypodiploidy <44 chromosomes.

Pediatric oncologists treating children and adolescents with ALL have pioneered the use of MRD to monitor response to treatment, and all major pediatric oncology centers and cooperative groups worldwide now systematically use MRD levels to guide treatment decisions (Table 1).[8-20] Because precise measurements of MRD have important prognostic and therapeutic implications, it is essential to understand their clinical significance in the context of presenting clinical and biologic features, treatment regimen, and time interval at which MRD is measured.

MRD-directed treatment of high-risk genetic subtypes of ALL (case 1)

Genetic abnormalities of leukemic lymphoblasts have prognostic significance and have been used to inform treatment decisions.[21] The genetic subtype defined by hypodiploidy (<44 chromosomes), especially near-haploidy (24-31 chromosomes), and low-hypodiploidy (32-39 chromosomes), has generally been associated with unfavorable prognosis.[22] Hence, hematopoietic stem cell transplantation (HSCT) in first remission is still offered to patients with hypodiploid (<44 chromosomes) ALL in many contemporary clinical trials.[23] However, treatment outcome of hypodiploid ALL and many other high-risk genetic subtypes of ALL is not uniformly poor because it depends on other leukemia cell variables (eg, cooperative genomic abnormalities, self-renewal capacity, drug resistance), host factors (eg, pharmacogenetics), and efficacy of postremission treatment regimen.[21]

Our Total Therapy Studies XV and XVI relied on MRD measurements for final risk assignment, an approach that may over-ride or lessen the prognostic impact of specific genetic abnormalities of leukemic cells.[17,24] Our assumption was that sequential MRD monitoring during remission induction therapy should detect heterogeneity in chemotherapy sensitivity of leukemia cells among patients with the same genetic subtype. If so, it should be possible to use this information to adjust treatment intensity and avoid over- or undertreatment. Accordingly, patients with hypodiploid ALL were prospectively assigned to receive intensive chemotherapy, and only those with MRD ≥1% at the end of remission induction were offered allogeneic HSCT as a treatment option. This strategy resulted in a 5-year event-free survival of 73.6% for the 20 patients with hypodiploid ALL and a 5-year event-free survival of 91.7% for the 13 who achieved MRD-negative status at the end of remission induction and were treated only with chemotherapy.[22] These data demonstrate that patients with hypodiploid ALL who have a good response to remission induction therapy, as indicated by achievement of MRD negativity, can be successfully treated with intensive chemotherapy alone. In our study, there were too few hypodiploid patients with positive MRD at the end of remission induction to conclusively determine whether allogeneic HSCT can improve their outcome.

In the era of MRD-directed therapy, further studies, preferably randomized, are needed to identify the optimal way to incorporate MRD monitoring into treatment strategies for hypodiploid ALL and other high-risk genetic subtypes of ALL, such as Philadelphia chromosome–positive (Ph^+; *BCR-ABL1*), Ph-like ALL, t(17;19)/*TCF3-HLF*, and intrachromosomal amplification of chromosome 21.[21] In the EsPhALL and Children's Oncology Group AALL0031 studies for Ph^+ ALL, treatment with intensive chemotherapy plus imatinib or allogeneic HSCT yielded comparable treatment outcomes.[25,26] Our data indicate that adding ABL tyrosine kinase inhibitor to remission induction chemotherapy in patients with this leukemia subtype can dramatically reduce the level of MRD at the end of remission induction.[27] Thus, HSCT in first remission is warranted only for patients with Ph^+ ALL who have positive MRD after intensive remission induction that includes an ABL tyrosine kinase inhibitor. In a recent retrospective study, we found that MRD-directed treatment used in our Total Therapy XV study also improved outcome for patients with Ph-like ALL.[28] In this trial, patients with high MRD at the end of remission induction received allogeneic HSCT, whereas those who achieved MRD-negative status at the end of remission induction (approximately 40%) received relatively low-intensity chemotherapy and had 5-year event-free survival of 100%.[28] Anecdotal studies suggest that the addition of ABL-class inhibitor (eg, imatinib) can improve outcome of patients with Ph-like ALL and ABL-class fusion who have poor response to remission induction.[29-32]

Recommendation

For children and adolescents with high-risk genetic subtypes of ALL who attain MRD-negativity (<0.01%) at the end of remission induction, we recommend proceeding with intensive chemotherapy, with the addition of an appropriate tyrosine kinase inhibitor in patients with Ph^+ ALL or Ph-like ALL with ABL-class fusion transcript.[33] More studies are needed to determine whether allogeneic HSCT or emerging experimental therapies can benefit patients with high MRD after remission induction treatment or persistent disease after consolidation treatment.

Specific leukemia subtypes remain prognostic in the context of MRD-directed treatment (case 2)

Children and adolescents with ALL who do not achieve morphologic remission after the initial 4-week course of chemotherapy (induction failure) have been regarded as having chemotherapy-resistant disease and should be considered as candidates for allogeneic HSCT. However, an international collaborative study showed that the prognostic impact of induction failure after conventional remission induction therapy was not uniform among different subtypes of ALL. Thus, despite induction failure, children with hyperdiploid (>50 chromosomes) B-cell ALL (B-ALL) had a relatively favorable 10-year survival of 71% ± 6% when treated with chemotherapy alone without transplantation.[20] This outcome was possibly the result of the known increased sensitivity of the blast cells to methotrexate and mercaptopurine, drugs that are generally used at low doses or not at all during remission induction but are used in high doses later.[20] Postremission chemotherapy was generally not as effective in patients with other subtypes of ALL; in those with T-cell ALL (T-ALL) and induction failure, allogeneic HSCT was more effective than intensive chemotherapy alone.[20]

Case 2 had T-ALL, with residual disease by flow cytometry of 62.9% on day 19 and 5.82% on day 46 of remission induction therapy. Because of poor early response to initial chemotherapy, he received allogeneic HSCT after achieving MRD-negative status with further chemotherapy. The Associazione Italiana Ematologia Oncologia Pediatrica Berlin-Frankfurt-Münster 2000 (AIEOP-BFM-ALL 2000) study used MRD levels on day 33 and day 78 of treatment of risk classification.[34] It found that the latter measurement was more informative for predicting relapse in T-ALL, with 21% of patients meeting the high-risk criterion of MRD ≥0.1% on day 78.[34]

These patients had a 7-year event-free survival of only 49.8%, significantly worse than that of patients with lower levels of MRD, particularly those with MRD <0.01% on day 33. In our Total Therapy XV study, even among patients with negative MRD on day 46, those with T-ALL had a poorer event-free survival (78.7%) and an inferior overall survival (86.4%) than did patients with other leukemia subtypes.[19]

With the increasing optimization of standard therapy and the availability of new agents for ALL, an ever more refined risk algorithm that combines presenting biologic and genetic features with MRD measurements is needed to develop optimal postremission treatment strategies.[19] We have shown that among patients with positive MRD at the end of remission induction, serial monitoring of MRD is important, because some patients may be cured with chemotherapy alone if MRD becomes undetectable after subsequent treatment.[19] For example, in early T-cell precursor (ETP) ALL, which is generally associated with high levels of MRD during and at the end of remission induction therapy,[35] recent studies suggest that postremission chemotherapy, such as consolidation treatment phase 1B of the AIEOP-BFM regimen with 2 courses of cyclophosphamide, mercaptopurine, and cytarabine might be effective in reducing MRD and could mitigate an adverse prognosis.[36,37] In this regard, MRD measured at later time points (eg, day 78 of AIEOP-BFM studies or week 14 of United Kingdom Acute Lymphoblastic Leukaemia (UKALL) studies; Table 1) should be particularly useful for identifying patients who have a high risk of relapse (ie, those who have residual disease after receiving adequate doses of most, if not all, potentially effective chemotherapeutic or targeted drugs).[19]

Recommendation

Children and adolescents with ALL who have high levels (≥1%) MRD at the end of remission induction therapy and persistent MRD at subsequent time points have a very high risk of relapse if treated with intensive chemotherapy alone. For these patients, we recommend allogeneic HSCT. Because MRD levels before transplantation are directly associated with risk of relapse posttransplant,[38] additional treatment directed at reducing levels of MRD before transplant should be considered. Some patients with high levels of MRD at the end of remission induction (eg, those with hyperdiploid [>50 chromosomes] ALL or ETP-ALL) may continue treatment with intensive chemotherapy without allogeneic HSCT if they achieve MRD-negative status after consolidation treatment.

Conclusion

MRD monitoring has redefined remission in ALL. Numerous studies have demonstrated the strong association between MRD levels and treatment outcome in childhood ALL,[5,6] supporting the concept that MRD during the initial phases of chemotherapy provides a reliable measurement of the drug sensitivity of leukemic lymphoblasts. This realization has profoundly refined risk-directed therapy, with MRD being applied in virtually all major protocols for pediatric ALL to guide treatment decisions.

Table 1 summarizes some of the risk classification guidelines used in current pediatric ALL trials in the United States and Europe. It is evident that there is no consensus on the precise time points at which MRD should be measured and on the levels used for treatment decisions. The algorithms are typically built on the experience of previous correlative studies by each study group, with the timing for MRD studies adapted to the treatment design and schedule in each individual protocol. The predictive value of MRD depends on the preceding and subsequent treatment and must be determined in the context of each treatment regimen. There are, however, some general principles that can be extrapolated from the published data and that are exemplified by the cases discussed here. Patients who have high levels of MRD (ie, ≥1%) at the end of remission induction therapy and persistent MRD after subsequent consolidation treatment have a very high risk of relapse if treated with currently available chemotherapy. The best treatment option for these patients at this point in time is allogeneic HSCT, particularly if levels of MRD can be reduced to undetectable status before transplant. Emergent immunotherapeutics might facilitate MRD reduction in these patients and could also be curative without further treatment.[39,40] Conversely, patients with high-risk presenting features can be cured with chemotherapy alone if they achieve MRD negativity (ie, <0.01%) at the end of remission induction or consolidation therapy, with the possible exception of those with t(17;19)/*TCF3-HLF*. To this end, MRD-guided therapy can improve the outcome of some high-risk groups of patients, such as older adolescents[41] and those with hypodiploidy,[22] or Ph-like ALL.[28]

The use of MRD in ALL relies on highly sophisticated methods and a detailed understanding of its clinical significance, evolving over 4 decades of basic, translational, and clinical research. Conceivably, newer methods that can detect MRD at lower levels than the standard threshold of 0.01% will further refine monitoring of treatment response.[7,42] With 5-year survival rates exceeding 90% in many developed countries,[33] current efforts are focused on the early identification of patients with highly curable leukemia to avoid short-term morbidity and mortality and long-term treatment-related sequelae.[12,13] In this regard, attainment of negative MRD after exposure to only a few drugs for a short duration of time (ie, 2 weeks from treatment initiation), is a very useful indicator.[10,43] This approach is particularly helpful in patients with t(12;21)/*ETV6-RUNX1* or hyperdiploid (>50 chromosomes) ALL.[19] However, its effectiveness depends on the intensity of subsequent treatment. The risk features and MRD time points to be used must be selected with caution. Thus, in a recent analysis of the AIEOP-BFM 2000 study, an increased relapse rate was observed for patients with B-ALL regarded as standard risk (defined primarily by leukocyte count and age) who had received reduced-intensity delayed intensification because of negative MRD on days 33 and 78.[44] With an expanding arsenal of agents for ALL, the application of MRD must be adapted so that novel treatment strategies can be designed effectively. Thus, MRD monitoring can contribute to the development of novel immunotherapeutic approaches by serving as an eligibility or response criterion.[39,40]

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Authorship

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