INTRODUCTION

The detection of leukemia cells above a specified threshold both during treatment and prior to allogeneic hematopoietic cell transplantation (HCT) has predicted an unfavorable outcome for children with advanced acute lymphoblastic leukemia (ALL) [1–10]. The evaluation of minimal residual disease (MRD) prior to HCT in ALL has been predominantly accomplished by polymerase chain reaction (PCR), requiring the creation of patient-specific primers on the diagnostic specimen to detect unique B-cell immunoglobulin sequences or T-cell receptor rearrangements. Several studies have concluded that the detection of MRD by PCR at time of allogeneic HCT in pediatric ALL correlates with inferior outcome [1–5,7,8,10,11]. With careful standardization of methodology, PCR and flow cytometry yield comparable results [11–14]. PCR testing requires procurement of specimens at diagnosis for the creation of the specific molecular probes. The technique is expensive, labor intensive, time consuming, and not uniformly available outside of large research institutions. Flow cytometry can provide an alternative to PCR.

Flow cytometric detection of residual disease during initial treatment in pediatric ALL is highly correlated with outcome, and is thus becoming a standard method for disease detection [11–14]. Residual disease detection following HCT in pediatric and adult ALL has also been associated with a high risk of recurrence [15–17]. However, like the PCR techniques, most flow cytometric approaches to residual disease detection are based on the phenotype identified at diagnosis, that is, the leukemia-associated immunophenotype [2]. Leukemia-associated immunophenotyping relies on the diagnostic specimen to dictate the gating strategy and then determination of residual disease based on that particular constellation of abnormalities. However, transplant centers may not have access to the diagnostic specimen. Consequently, the detection of abnormal leukemic blasts based on “difference from normal” as a tumor-specific marker may be more widely applicable to identify residual tumor without the need for a diagnostic references [16,17]. Furthermore, the emergence of leukemic sub-clones or changes in immunophenotype after treatment using leukemia-associated immunophenotyping approach may hamper the detection of these abnormal leukemic sub populations. Finally, the FCM approach of using “difference from normal” has been shown to be more specific and more sensitive than either morphology or cytogenetics or the two combined to predict outcome post hematopoietic stem cell transplant [16].

METHODS

Using a retrospective IRB approved study, we evaluated 116 patients who underwent myeloablative HCT for relapsed or very high risk ALL between April 1995 and December 2005 at Seattle Children’s Hospital, the pediatric hospital serving the Fred Hutchinson Cancer Research Center in Seattle, WA. All patients underwent marrow evaluation, including morphology and multidimensional flow cytometry, within 30 days prior to HCT. All patients were in complete morphologic remission (fewer than 5% by conventional pathology) prior to HCT. Analysis was performed at the time of sampling by three color flow cytometry patterning of normal antigen expression and detecting small populations of abnormal cells as previously described [16,17]. The following reagents were used for B lineage ALL: CD10, CD19, CD20, CD22, CD34, CD38, and CD45 and for T lineage: CD1a, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD34, CD56, and CD45. Flow cytometric analysis was performed on either a Cytoron, Ortho Diagnostics (Raritan, NJ) or a FACS Calibur (Becton

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BRIEF REPORT

Minimal Residual Disease Detected Prior to Hematopoietic Cell Transplantation

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Previous studies to evaluate minimal disease in acute lymphoblastic leukemia (ALL) after treatment have relied on the diagnostic specimen to develop patient-specific analytical probes. The diagnostic specimen is often not available in a tertiary setting; therefore, we evaluated the use of flow cytometry (FCM) using a “difference from normal” approach to detect residual disease prior to myeloablative allogeneic hematopoietic cell transplantation (HCT). Among 116 pediatric patients with ALL who were in morphological remission at time of transplant, we found that those patients who had detectable residual disease by FCM prior to HCT experienced significantly inferior outcome. Pediatr Blood Cancer 2011;57:163–165. © 2011 Wiley-Liss, Inc.

Key words: ALL; molecular diagnosis and therapy; minimal residual disease; transplantation

The role of flow cytometry to detect residual disease in pediatric patients with ALL in morphological remission prior to transplant, has not been previously evaluated. We report a large single institution experience with tumor detection by flow cytometry prior to allogeneic ablative HCT over a 13-year period.
Dickinson BioSciences, San Jose, CA). Data analysis was performed using WinList (Verity Software House, Topsham, ME). The instruments were standardized daily using fluorescent microspheres, RFP-1, RCP-1; Spherotech, Lake Forest, IL. Residual disease was defined as a population of lymphoblasts (>0.1%) with abnormal relationships or intensities (>0.5 log units) that were discrepant from normal [16]. No diagnostic specimens were available for comparison.

All 116 transplant regimens consisted of total body irradiation based (1320 cGy) with either cyclophosphamide (n = 111), fludarabine (n = 2) or melphalan (n = 3). Statistical analysis of event-free survival (EFS) was performed using the Kaplan–Meier method for calculating survival rates and 95% confidence intervals (CI) [18]. EFS was defined as the time from transplant to either leukemia recurrence or death from any cause. Patients who were either still living or had not developed relapse were censored at the date of last contact. Differences in EFS among groups defined by patient or treatment characteristics were analyzed using the log-rank test [19]. EFS data current as of February 4, 2010 were analyzed using SPSS for Windows version 13.0 (SPSS Inc, Chicago, IL).

RESULTS

One hundred and sixteen patients underwent ablative HCT. Patient details are listed in Table I. Patients’ age ranged from 0.57 to 20.50 years, with a median age of 8.93 years. Of the patients evaluated, 98 patients were MRD negative by FCM at time of HCT compared to 18 patients who were MRD positive. The 5-year EFS for the entire group was 51% (95% confidence interval, 42–60%). For the group as a whole, the EFS for MRD positive and negative patients was 11% and 58%, respectively, Figure 1 (P < 0.001). MRD positive status at time of transplant was associated with lower EFS for patients who received a matched unrelated donor (URD) transplant (P = <0.001), as well as for patients who received matched sibling donor (MSD) transplants (P = 0.045). There was no difference in outcome based upon immunophenotype. The 5-year EFS for patients in first complete remission (CR1), second complete remission (CR2), and third complete remission (CR3) were 77%, 40%, and 29%, respectively. Residual disease detection was only significant with respect to remission status amongst patients in CR2 or CR3 (P = 0.001 and P = 0.013, respectively). Donor type (URD vs. MSD) did not impact EFS and there was no significant difference in non-relapse mortality between donor types and MRD (data not shown). Patients who were MRD positive had a significantly increased relapse rate compared with MRD negative patients (75% vs. 16%, P < 0.001). There was no significant differences in deaths from complications among the two groups (MRD positive, 30%; MRD negative 21%, P = 0.91).

DISCUSSION

The patient cohort presented here in morphological remission with MRD >0.1% detected pre-transplant had a poor EFS due to relapse post transplant. For those patients transplanted in CR2 or CR3, the presence of MRD has a significantly greater negative outcome compared with those patients in CR1. The difference in 5-year EFS was due to an increased relapse rate in MRD positive patients, not due to differences in deaths from complications of transplants. Using an URD source did not impact EFS. Among patients who underwent URD transplant, the presence of MRD was associated with inferior outcome. The lack of significance amongst patients in CR1 may reflect the low number of patients in CR1 who were MRD positive. Further studies using multivariate analysis with a larger population of patients are needed to deter-

| TABLE I. Patients |
|-------------------|
| N (%) | 5-year EFS (95% confidence interval) | P-value |
| All patients | 116 | 51% (42–60) | <0.001 |
| MRD status | | | |
| MRD+ | 18 (16) | 11% (0–26) | |
| MRD− | 98 (84) | 58% (53–63) | |
| Stem-cell donor type | | | |
| URD | 78 (67) | 53% (36–70) | |
| MRD+ | 13 (17) | 8% (0–23) | <0.001 |
| MRD− | 65 (83) | 59% (47–71) | |
| MSD | 38 (33) | 51% (40–62) | |
| MRD+ | 5 (13) | 20% (0–56) | 0.045 |
| MRD− | 33 (87) | 56% (38–74) | |
| Immunophenotype | | | |
| BCP | 99 (85) | 49% (39–59) | 0.71 |
| T-lineage | 8 (7) | 63% (29–97) | |
| Remission status | | | |
| CR1 | 39 (34) | 77% (64–90) | |
| MRD+ | 2 (5) | 50% (0–100) | 0.41 |
| MRD− | 37 (95) | 78% (64–92) | |
| CR2 | 64 (55) | 40% (28–52) | |
| MRD+ | 13 (20) | 8% (0–23) | 0.001 |
| MRD− | 51 (80) | 48% (34–62) | |
| CR3 | 13 (11) | 29% (3–55) | |
| MRD+ | 3 (23) | 0% (0–71) | 0.013 |
| MRD− | 10 (77) | 38% (6–70) | |

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mine the relationship between FCM results and other features of ALL including genetic subtype, CR duration, and age.

This study demonstrates that FCM analysis prior to HCT can be performed to identify a blast population without access to the diagnostic specimen. By quantitative analysis of the antigens expressed on regenerating normal B lymphoid precursors, it is possible to identify aberrant cells associated with residual disease [16,20]. The FCM process has been shown to have equivalent ability to detect residual disease as slower and more costly, PCR-based methods [11–13]. The FCM detection requires precise quality control of both the FCM process and analysis, resulting in the detection of low levels of tumor even in stressed marrows post chemotherapy. Three-color (FCM) was adopted rather than four or more-color FCM because it is less expensive and time consuming than a multi-dimensional analysis, and there is a higher reproducibility in a high volume clinical practice.

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Fig. 1. Event-free survival.