Detection of engraftment, chimerism and minimal residual disease in BMT

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Following allogeneic bone marrow transplantation (BMT) careful monitoring of engraftment is important because the finding of residual host cells may precede the reappearance of the overt abnormal hematopoietic clone. Classic cytogenetics for detection of X and Y chromosomes in bone marrow cells from sex-mismatched transplant recipients is labor-intensive, time consuming and limited by the number of cells that can be analyzed. To exclude mosaicism at the level of 1%, 300 or more metaphase cells must be examined. Obtaining this many metaphase cells from bone marrow following transplantation is nearly impossible in a routine cytogenetic laboratory. The chief advantage of cytogenetics, however, is unambiguous detection of leukemic cells. Other methods of detecting and monitoring chimerism are based on population studies and have either poor sensitivity, a high rate of false positivity, or unproven biological or clinical significance.

Fluorescence In Situ Hybridization (FISH) allows the rapid screening of large number of cells and is becoming a powerful tool for monitoring engraftment with a high sensitivity and low false positivity rate.

DETECTION OF ENGRAFTMENT

In 1997 we reported study on utilizing dual color XY probes to detect engraftment and chimerism on 27 patients following allogeneic sex mismatched BMT (1). In this presentation I will extend the original report and present our findings on 57 patients who were evaluated, using XY interphase FISH test between July 1994 and May 1999.

PATIENTS IN CONTINUOUS REMISSION

Bone marrow and/or peripheral blood was investigated in 29 of 57 studied patients who underwent allogeneic sex mismatched BMT. Twenty-one patients received bone marrow -related cells, seven patients received bone marrow unrelated cells and one patient received cord unrelated blood cells. Twenty-three patients had leukemia, three patients had severe aplastic anemia, and one patient each had paroxymal nocturnal hemoglobinuria, familial hemophagocytic lymphohistiocytosis, and Waldenstrom's macroglobulinemia. In each patient a minimum of 1,000 nuclei were scored in each test for the presence of XX or XY status. Post-transplantation results from 103 specimens (over 150,000 evaluated nuclei), from day 14 to day 3,388 (9 years) showed between 0 and 2.4% of host cells. The mean number of host cells for all 29 patients was 0.28% ± 0.4%. As shown below twice as many host cells were detected in marrow than in peripheral blood between 4 weeks and 9 years post allogeneic BMT.

Tissue	Day 14-28		Day 29-3388	
	No. of Samples	Host Cells % + s.d(range %)	No of Samples	Host Cells % + s.d. (range %)
PB	8	0.47 ±0.4 (0-1.4)	46	0.17 ± 0.4 (0-1.5)
BM	6	0.30 ± 0.3 (0-0.9)	32	0.33 ± 0.45 (0-1.1)

In the last two years of follow up study, these small numbers of residual host cells were not predic-

tive of relapse. Michrochimerism has been reported in other studies and relapse has not been found using highly sensitive polymerase chain reaction (PCR) studies (2).

Additional 14 patients (23 samples) engrafted but expired between day #13 and day #203. Because a long-term follow up could not be performed for this group of patients they are discussed as a separate group. Four patients received marrow -related transplant, two patients received marrow unrelated transplant, four patents received peripheral blood progenitor related cells, and four patients received cord blood unrelated transplant. Eight patients had leukemia, one patient had breast cancer, two patients had lymphoma, one patient each had thalassemia, severe combined immunodeficiency syndrome and spent phase of polycythemie vera. The mean number of host cells for all 23 specimens was 0.13% ± 0.3%. When examined day 10 to day 28, the mean number of host cells in 10 peripheral blood specimens was 0.01%± 0.02%. From day 29 to day 140, the mean number of host cells in 6 bone marrow specimens was 0.24% ± 0.3% and in five peripheral blood specimens 0.01%± 0.03%. From the hematological and molecular cytogenetics point of view these 14 patients did not differ from 29 patients who were in continuous remission up to 9 years.

PATIENTS WHO FAILED TO ENGRAFT OR RELAPSED

Detection of engraftment was performed on 14 patients (114 specimens) who either did not engraft or relapsed. Three of these patients underwent the second transplantation from different donors. Eight patients received bone marrow related transplant, two patients received bone marrow unrelated transplant, two patients received peripheral blood progenitor related cells, and two patients received cord peripheral blood unrelated transplant. Seven of the 14 patients are alive 60 to 3,129 days following transplantation and donor lymphocyte infusion. In all 14 patients host cell's genotype was detected.

DETECTION OF CHIMERISM

A comparison of FISH results obtained from peripheral blood and bone marrow on the same day demonstrated that routine studies of patients who are in hematological remission (9 patients, 14 specimens) could be performed using peripheral blood specimens alone (BM: 0.2% versus PB: 0.3%). In sharp contrast, 9 of 20 specimens (45%) from 12 patients who failed to engraft or were in relapse showed a substantially higher percentage of host cells in bone marrow than in blood. Seven of these 9 specimens were from patients with AML and two were from one patient with CML. The differences were striking. For example, one patient with AML had on day 28 following BMT 48% of host cells in the bone marrow and 2.6% host cells were found in peripheral blood. Other patients had similar distributions. These observations suggest that bone marrow must be the primary tissue for monitoring engraftment for this group of patients.

DETECTION OF MINIMAL RESIDUAL DISEASE (MRD)

The significance of residual disease following BMT for CML and AML may be different depending on the technique and the interpretation of results. Cytogenetics analysis has a low sensitivity and will miss BCR-ABL+, Ph-negative patients as well as some patients with "dormant" monosomy 7. There appears to be a consensus that when cytogenetic abnormality is present clinical relapse is inevitable. Although in many centers polymerase chain reaction (PCR) is being used, the interpretation and published data are controversial. The information from the published reports strongly suggests that many patients with CML and some with AML have evidence of minimal residual disease when sensitive technique, such as PCR is applied. The question that was recently raised is whether positive PCR always equals relapse? PCR is a powerful tool but has a number of shortcomings that can lead to false positive or false negative results. Some of the problems relates to technical pitfalls as well as the choice of primers, subclone formation and lack of PCR quantitation. Recently, Faderl and colleagues published a commentary in which they cited a multiple evidence where positive PCR reaction did not always equal relapse and a negative PCR reaction did not equal cure (3)

For detection of minimal residual disease in patients with chronic myelogenous leukemia (CML) who received sex-mismatched BMT, we used interphase FISH (I-FISH) study with dual color XY and dual color BCR-ABL. Nine patients (45 specimens) with CML who received sex mismatched allogeneic BMT were followed between day 26 and day 3282 for the presence of host cells (XX vs. XY) and for the BCR-ABL fusion positive cells in XX or XY cells. Seven patients were in continuous remission and two patients relapsed.

Between 0% and 1% of host cells (XX vs. XY) were detected in 29 specimens from patients who were in continuous remission. None of the residual host cells had BCR-ABL fusion whereas between 0% and

4% of BCR-ABL fusion positive cells were found only in donor cells. The normal laboratory reference range for BCR-ABL was up to 5% (first generation of probe). These patients are in continuous remission up to 9 years.

Using this sensitive method in two patients who relapsed we were able to distinquish host cells with and without BCR-ABL fusion allowing the quantitation of normal and abnormal host cells. Furthermore, one patient was serially followed after she received donor lymphocyte infusion. The patient relapsed on day #105 following BMT when peripheral blood showed 60% cells with host genotype and 52% host cells with BCR-ABL fusion. Following the donor lymphocyte infusion and sequential I-FISH study (11 specimens), on day #190, the peripheral blood cells showed 0% host cells and 1.4% BCR-ABL fusion detected in donor genotype cells.

Although FISH technology is not as sensitive as PCR (10^3 versus 10^6), it may be more clinically meaningful since it detects genotype on a cell by cell basis.

To determine whether I-BCR-ABL may be used to determine MRD for patients who received sex-matched allogeneic BMT we studied 64 specimens from 23 patients (22 with CML and 1 with ALL), who were 100% Ph-positive at diagnosis and in confirmed remission up to 9 years. The mean number of BCR-ABL fusion positive cells was 2.8% + 2.0% and a mean number of normal cells was 96.3% + 3.0%. Since the of BCR-ABL fusion positive cells is within a normal laboratory reference range, these patients are considered BCR-ABL FISH negative.

With the development of more refined probes, we recently used centromere-enumeration probe for chromosomes 7, 8, and locus-specific probe for chromosome 21 to detect MRD in 4 patients with MDS and JMML. In a patient with MDS, who had trisomy and tetrasomy of chromosome 8 and 21 at diagnosis, on day #287 following BMT, the bone marrow I-FISH showed 8% of cells with trisomy and tetrasomy for chromosome 8. By day #352, following donor peripheral blood infusion, dual color I-FISH showed 14% cells who had trisomy and tetrasomy of chromosomes 8 and 21: 4% of cells had trisomy for chromosomes 8 and 21 in the same cell and 10% cells showed tetrasomy for these abnormalities in the same cell. These findings illustrates the advantage of I-FISH in its ability to detect MRD on a cell by cell basis. Similarly, the use of chromosome 7 was not only successful in detecting MRD in twins with JMML, but also in determining that the marker chromosome found in these twins was a partial monosomy 7 and had, most likely, an embryonic origin (4). Very recently, we used TEL-AML-1 probe for detection of t(12;21) in patients with childhood ALL. We found that 30-40% of pediatric patients with pre-B-cell ALL have amplification (4-15 copies) of AML-1 gene. One patient had 80% of cells at diagnosis that showed up to 15 copies of AML-1 gene. Follow-up studies demonstrated a normal AML-1 distribution in remission, but when and 19% of cells had with multiple AML-1 copies when relapse occurred. The patient received BMT, had transient engraftment and expired in peri-transplant period. These observations suggest that in future detection of minimal residual disease using I-FISH may be individually tailored on the basis of any abnormal gene found at diagnosis.

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