Consultations in Molecular Diagnostics

Cytogenetic Changes Associated with Myelodysplastic Syndrome Affecting Bone Marrow Engraftment Analysis

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***In vitro* amplification of polymorphic genetic markers, especially short tandem repeats (STRs), has become standard laboratory practice in the monitoring of allogeneic bone marrow transplant patients. After initial analysis of donor and recipient samples at multiple loci before transplantation, one or more loci are used to follow engraftment status in subsequent specimens. We describe an unusual pattern of STRs in a transplanted patient with a prior history of refractory acute myelogenous leukemia. DNA chimerism studies showed a lack of engraftment at 1 and 2 months after transplantation. Atypical minor peaks occurred at each of three STR loci in the pre-transplant and 2-month post-transplant recipient samples. However, these peaks were of equal amplitude as the major corresponding allele in the 1-month post-transplant sample. A history of myelodysplasia with specific chromosomal deletions before the patient's acute myelogenous leukemia diagnosis appears to explain the spurious peaks. STR analysis of blood and archival paraffin-embedded tissues collected from the patient at various time points before transplantation reflected the evolution, progression, and response to therapy of the myelodysplasia. The case illustrates the need for comprehensive evaluation of pertinent clinical and laboratory data during engraftment monitoring to identify potential sources for error in interpretation of STR analysis. (J Mol Diagn 2006, 8:288–294; DOI: 10.2353/jmoldx.2006.050097)**

laboratory procedure used in the management of patients receiving allogeneic bone marrow transplants.^{1,2} However, the choice of loci to be examined largely remains laboratory specific. Several commercially available kits, targeting either multiple short tandem repeats (STRs) or variable number of tandem repeats (VNTRs), have been used extensively for human identification purposes in the forensic community. These kits have also been applied to engraftment monitoring of patients after allogeneic bone marrow transplantation. In addition, a number of “home-brew” monoplex or multiplex amplification assays using STRs, VNTRs, or other loci have been developed for engraftment monitoring.³ Previously, Southern blot hybridization was the only nucleic acid-based technology available for these analyses; however, the majority of these assays are now based on amplification technologies.

The general application of these assays for allogeneic bone marrow engraftment monitoring or chimerism studies involves initial analysis of DNA, obtained from buccal swabs or peripheral blood from the donor and recipient before the transplant procedure, in an attempt to identify at least one informative locus that can be used to discriminate the recipient from the donor. Then at various time points after transplantation, peripheral blood and/or bone marrow samples from the recipient are analyzed at the previously identified informative locus. Post-transplant sample collection is generally performed on day 21 or 30, day 60, and day 90 and then as clinically indicated. Relative amplification of donor and recipient alleles (as determined from peak heights or areas under the peaks) is then used to assess the relative cellular contributions from the donor and recipient in post-transplant samples. Commonly, once an informative locus or loci have been established for a donor/recipient pair, these are specifically targeted to determine the transplantation status of

Bone marrow engraftment monitoring using polymerase chain reaction (PCR) amplification targeting various polymorphic loci followed by capillary electrophoresis of fluorescently labeled PCR products has become a standard

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the patient in subsequent specimens submitted to the laboratory.

Materials and Methods

Case Description

A 43 year-old Caucasian woman diagnosed with refractory secondary acute myelogenous leukemia (AML) was admitted to the OU Medical Center for an allogeneic cord blood transplant. The patient had failed to achieve hematological remission with induction therapy consisting of idarubicin and cytarabine (7 + 3) and re-induction with high-dose cytarabine arabinoside around 2 weeks later. Her bone marrow showed an increasing number of myeloblasts, reaching 85% 4 months later. In the absence of a human leukocyte antigen (HLA)-matched sibling or an unrelated bone marrow donor, she received a sex-matched, umbilical cord blood transplant (cell dose = $2.08 \times 10^7/\text{kg}$; five of six HLA antigens matched). Her conditioning regimen consisted of cyclophosphamide at 60 mg/kg/day for 2 days and total body irradiation with a total dose of 12 Gy over 4 days and anti-thymocyte globulin at 15 mg/kg/day. Graft-versus-host disease prophylaxis included a short course of methotrexate (days 1, 3, and 6 at 5 mg/m²) and cyclosporine dosing. Peripheral blood counts remained low after transplantation with total white blood cell count of $<0.4 \text{ K/mm}^3$ (normal = 4.0 to 11.0 K/mm³), hemoglobin of 10.9 g/dl (normal = 12.0 to 16.0 g/dl), and platelet count of 18 K/mm^3 (normal = 140 to 440 K/mm³) on day 31. Pathological examination of bone marrow (BM) aspirate and biopsy performed on day 31 revealed a severely hypocellular marrow with no evidence of engraftment and rare myeloblasts. Repeat bone marrow biopsy on day 54 demonstrated a hypocellular marrow with abnormal hematopoiesis and clusters of myeloblasts. A chronology of clinical events for this patient and specimen acquisitions for DNA chimerism studies is provided in Figure 1.

DNA Chimerism Studies

Pre-transplant recipient peripheral blood (PB) and unrelated donor umbilical cord blood samples were received, as part of DNA chimerism studies, 8 days before transplantation. Patient samples were subsequently received 31 days (PB) and 54 days (BM) after transplantation. DNA was extracted from samples using the QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA) per the manufacturer's instructions. Samples were tested using three separate *GenePrint* Fluorescent STR Multiplex Systems (CTTv, FFFL, and GammaSTR kits) as indicated by the manufacturer (Promega Corporation, Madison, WI). Each of these kits includes primers that amplify four separate STR loci, each consisting of variable tetranucleotide repeats, in a multiplex reaction. Briefly, each PCR reaction contained 2.5 μl of Promega STR 10 $\times$ buffer, 2.5 μl of 10 $\times$ Primer Pair Mix, 1 U of Promega *TaqDNA* Polymerase in Storage Buffer B, 10 ng of DNA, and sterile water to a final volume of 25 μl . PCR was performed in a Cetus

Years Months

-7	Diagnosis of essential thrombocytopenia
-5	Gall bladder surgery
-2	Diagnosis of MDS
-1	Skin biopsy – erythema multiforme
0	Diagnosis of AML – BM biopsy and cytogenetics
1	1st induction chemotherapy - failed
2	2nd induction chemotherapy - failed
5	HLA blood sample
6	Pre-transplant blood sample
	BM transplant
7	+31 days – BM biopsy and molecular chimerism studies
8	+54 days – BM biopsy and molecular chimerism studies
10	Patient deceased

Figure 1. Chronology of salient clinical events and sample collections from time of diagnosis of ET to death of patient. Times are given relative to the diagnosis of AML.

GeneAmp PCR System 9600 (Perkin-Elmer, Norwalk, CT) using the following conditions adapted from Promega's recommendations: 96°C for 1 minute; 10 cycles of 94°C for 1 minute, ramp 68 seconds to 60°C and hold for 30 seconds; ramp 50 seconds to 70°C and hold for 45 seconds; 20 cycles of: 90°C for 30 seconds, ramp 60 seconds to 60°C and hold for 30 seconds; ramp 50 seconds to 70°C and hold for 45 seconds; 60°C for 30 minutes; and 4°C hold. After amplification, 1 μl of PCR product was mixed with 23.5 μl of deionized formamide and 0.5 μl of Fluorescent DNA Ladder (CXR; Promega Corporation) as an internal size standard. Fluorescently labeled reaction products were detected using an ABI PRISM 310 Genetic Analyzer (3-second injection, 15 kV, GS STR POP4 (1 ml) A module, 60°C, 26-minute run time) with a 47-cm $\times$ 50- μm capillary and Performance Optimized Polymer 4 (Applied Biosystems, Foster City, CA). GeneMapper software (Applied Biosystems) was used to size PCR products generated from the *GenePrint* Fluorescent STR Systems. Relative contributions of donor and recipient cells were determined using calculated areas under informative allelic peaks.

Results

Nine loci were determined to be informative out of a total of 12 examined; however, in 4 of these informative loci, the donor and recipient shared common alleles and were separated by only 1 repeat, making calculations to determine donor/recipient contributions in the post-transplant sample only semiquantitative (Table 1). The vWA locus (on chromosome 12) was selected as the optimal informative locus to make quantitative assessments of recipient alleles in the post-transplant samples. The patient had a bi-allelic pattern at this locus with peaks at 14 and 15 repeats, whereas the donor demonstrated a single peak at 16 repeats (Figure 2, A and B). The presence of a recipient-specific peak at least 2 repeats smaller

Table 1. STR Analysis of Donor and Patient Prior to Bone Marrow Transplantation and on Days 31 and 54 after Transplantation

Kit	Chromosome location	Locus	Donor peaks	No. of repeats		
				Recipient		
				Pre-transplant peaks (-8 days transplantation)	Post-transplant peaks (% recipient) (31 days transplantation)	Post-transplant peaks (% recipient) (54 days transplantation)
FFFL	8p22	LPL	9	9, 11	9, 11 (85; semiquantitative)	ND
	1q31-q32.1	F13B	7, 9	7, 9	7, 9 (noninformative)	ND
	15q25-qter	FESFPS	9	9, 10	9, 10 (91; semiquantitative)	ND
	6p24.3-25.1	F13A01	4, 5	5, 6*	5, 6 [†] (100)	5, 6 [‡] (100)
CTTv	12p12-pter	vWA	16	14, 15	14, 15 (100)	14, 15 (100)
	11p15.5	TH01	5, 6	6	5, 6 (noninformative)	5, 6 (95; semi-quantitative)
	2p25.1-pter	TPOX	7	7, 10	7, 10 (93; semiquantitative)	7, 10 (96; semi-quantitative)
	5q33.3-34	CSF1PO	9, 11	10 [§] , 11	10 [¶] , 11 (100)	10 , 11 (100)
GammaSTR	5q23.3-32	D5S818	10	8**, 9	8 ^{††} , 9 (100)	ND
	13q22-q31	D13S317	11	11, 12	11, 12 (76; semiquantitative)	ND
	7q11.21-22	D7S820	10	8, 9	8, 9 (100)	ND
	16q24-qter	D16S539	10, 12	10, 12	10, 12 (noninformative)	ND

*This recipient peak was 26% size of peak at five repeats.

†This recipient peak was the same size as peak at five repeats.

‡This recipient peak was 29% size of peak at five repeats.

§This recipient peak was 24% size of peak at 11 repeats.

¶This recipient peak was the same size as peak at 11 repeats.

||This recipient peak was 37% size of peak at 11 repeats.

**This recipient peak was 15% size of peak at nine repeats.

††This recipient peak was the same size as peak at nine repeats.

Three kits were used to analyze 12 separate STR loci on different chromosomes. ND, not done.

than the donor-specific peak and the presence of two unique recipient-specific peaks made the choice of this locus optimal.¹ However, selection of an optimum locus for chimerism analysis of this patient was restricted to loci that would generally be regarded as having low sensitivity. Calculations using this locus indicated that there were 100% recipient cells in the 31- and 54-day post-transplant samples (Table 1).

Anomalous allelic patterns were noted for STR loci at F13A01 (on chromosome 6), CSF1PO and D5S818 (both on chromosome 5). On initial examination, peaks appeared to be present in the post-transplant sample that could not be readily assigned as recipient- or donor-specific. Figure 3A (panels 1 and 2) shows that the donor and recipient shared one peak (at 5 repeats) at the F13A01 locus. The donor also had a peak at 4 repeats at this locus. In the recipient's pre-transplant sample, a minor peak at 6 repeats, which was 26% of the area represented by the major allelic peak at 5 repeats, was also present, but the origin of this peak was initially unclear because of its smaller relative size. These same peaks (at 5 and 6 repeats) were the only peaks present in the 31- the 54-day post-transplant samples; however, in the day-31 sample, they were of equal size, whereas in the day-54 sample, the area of the 6-repeat peak was again diminished and was only 29% of the 5-repeat peak.

At the CSF1PO locus, the donor and recipient shared one peak (at 11 repeats), whereas a peak at 9 repeats was present in the donor but not the recipient (Figure 3B, panels 1 and 2). In the patient's pre-transplant sample, a minor peak was noted at 10 repeats at the CSF1PO locus, but this was only 24% of the 11-repeat peak. On initial analysis, it was unclear whether this minor peak was a true recipient peak or artifact of amplification. In both the day-31 and -54 post-transplant samples, these two peaks

(10 and 11 repeats) were the only peaks evident at this locus. However, in the day-31 sample, these peaks were of equal height and area, whereas in the day-54 sample, the area of the 10-repeat peak was diminished to 37% relative to the 11-repeat peak.

A similar skewed pattern, with a minor peak at 8 repeats and a major peak at 9 repeats was present in the recipient pre-transplant sample at the D5S818 locus. The minor peak represented 15% of the major peak in this sample (data not shown), leading to concern about its true identity. By contrast, these peaks were the only peaks present in the 31-day post-transplant sample (the donor-specific 10-repeat peak being absent) and were of similar amplitude and area. The 54-day post-transplant sample was not analyzed for this locus.

Discussion

On initial analysis of the post-transplant samples of this patient using the most informative locus, vWA, we determined that there was little or no engraftment at 31 and 54 days. The DNA chimerism studies were consistent with pathological examination of bone marrow aspirate and biopsies at these time points, demonstrating severe hypocellularity, no evidence of engraftment, and rare (31 day) or clustered (54 day) myeloblasts. Other STRs were run concurrent with the vWA locus and gave similar results. However, we noted atypical patterns of amplified products for two STR loci (F13A01 and CSF1PO) in the pre-transplant and the 54-day post-transplant samples and in D5S818 for the pre-transplant sample (day-54 sample was not analyzed for this locus). Each of the loci revealed one major peak (of similar amplitude and area as other peaks at other loci) and a minor peak which was

Figure 2. Capillary electropherograms showing amplification peaks for the vWA STR locus in the donor and recipient pre-transplant and recipient 31-day and 54-day post-transplant samples. The donor is homozygous with both alleles at 16 repeats. The patient has two recipient-specific peaks at 14 and 15 repeats in the pre-transplant and post-transplant samples, and these are of similar amplitude and area.

severely diminished in amplitude and area: in the pre-transplant sample, 26% in F13A01, 24% in CSF1PO, and 15% in D5S818, relative to the major allele; and in the 54-day post-transplant sample, 29% in F13A01 and 37% in CSF1PO, relative to the major allele. By contrast, the same sized peaks as those found at both loci in the pre-transplant and 54-day post-transplant samples were also present in the day-31 samples, but the peaks were of equal amplitude and area at each locus and did not show the skewed pattern. Typically, for any bi-allelic locus that has alleles differing by 1 repeat (4 bp), the resultant allelic peaks would be expected to be of similar height and area; however, these minor peaks were only one-fourth the area of the corresponding major peaks at each locus. Therefore, the exact origin of these minor peaks was initially unclear; were they truly recipient-specific peaks or amplification artifacts?

Occasionally, alleles will produce "stutter peaks" that are 1 repeat smaller ($N - 1$) or (less frequently) greater ($N + 1$) in size than the main allelic peak. These stutter peaks are most likely due to "slippage" of DNA polymerase during amplification of the repetitive STR sequence. In our experience, these stutter peaks are typically about 5 to 10% of the main peak. Because the minor peaks were 15 to 25% of the major peaks in the recipient pre-transplant and 54 day samples, they were not viewed as typical of $N - 1$ or $N + 1$ stutter.

In addition to stutter peaks, nonspecific peaks, which are occasionally produced during amplification, can

compromise the use of certain loci.¹ So, were these non-specific peaks? These minor peaks were not present in the donor sample for either locus, and all reactions (donor, recipient, and post-transplant samples) were run concurrently, so it was considered unlikely that these minor peaks were artifacts of nonspecific priming during amplification. Also, because donor-specific peaks for F13A01, CSF1PO, and D5S818 loci were absent in both post-transplant samples, the minor peaks were most likely contributed by the patient.

So, why should these minor peaks apparently resolve in the 31-day post-transplant samples relative to that observed in the pre-transplant sample and again emerge in the 54-day post-transplant sample? A review of the clinical history of the patient revealed that her diagnosis of AML was preceded by myelodysplasia (MDS) for 2 years and a diagnosis of essential thrombocytosis for 7 years (Figure 1). Cytogenetic analysis had been performed at the time of AML diagnosis, which was 6 months before transplantation. Examination of 20 metaphases showed monosomies and deletions of chromosomes in 19 cells and a normal karyotype in one cell; composite karyotype 45,XX,-5,del(5)(q13q31),del(6)(p21.3),-9,del(17)(p11.2)[cp]. The karyotypes of individual clones were not indicated in the report, although a descriptive text referred to the frequency of some specific deletions. An interstitial deletion of the long arm of chromosome 5 at breakpoints 5q13 and 5q31 [del(5)(q13q31)] and missing chromosomes 5 (-5) or 9 (-9) were observed in some abnormal cells. By contrast, terminal deletions of 6p [del(6)(p21.3)] and 17p [del(17)(p11.2)] were noted in all abnormal cells. It is worth noting that 5q deletions are commonly associated with AML and MDS, but 5q deletions were not observed in all of the abnormal cells in this case. By contrast, deletions of 6p and 17p were seen in every abnormal cell; therefore, the latter chromosomal changes likely represent the initial events in the disease process for this patient.

These cytogenetic changes underlying the patient's MDS and subsequent AML before transplantation were strikingly reflected in the results of the DNA chimerism studies. The monosomy 5 that was seen in a portion of cells would effectively remove one of the alleles at each of CSF1PO and D5S818 and produce a skewed pattern for these loci. One allele was observed to be 24 and 15% of the other allele, respectively, in the patient's pre-transplant sample by DNA analysis (Figure 2B). Because the CSF1PO locus resides at 5q33.3-34, the interstitial deletion at del(5)(q13q31) that was also observed in some of the patient's cells would not affect this locus; however, this interstitial deletion would affect the D5S818 locus that resides at 5q23.3-32 (Table 1). This may account for the slightly decreased amount of the minor allele at D5S818 (15%) relative to the minor peak at CSF1PO (24%). By contrast, the 6p deletion [del(6)(p21.3)] that was observed in each abnormal cell would be expected virtually to eliminate the signal for one of the F13A01 alleles. Consistent with this, one allele for this locus was calculated to be present at only 26% relative to the other allele in the patient's pre-transplant sample by DNA chimerism studies.

Figure 3. Capillary electropherograms showing amplification peaks for the F13A01 (A) and CSF1PO (B) STR loci in the donor and recipient pre-transplant and recipient 31-day and 54-day post-transplant samples. **A:** A minor peak at 6 repeats is present in the pre-transplant and 54-day post-transplant samples for the F13A01 locus, but this peak is of equal amplitude and area as the other peak in the 31-day post-transplant sample. **B:** Similarly, a diminished peak at 10 repeats is present in the pre-transplant and 54-day post-transplant samples for the CSF1PO locus, but the corresponding peak is of equal amplitude and area as the other peak in the 31-day post-transplant sample.

We can only speculate as to why the relative ratio of the alleles at F13A01, CSF1PO, and D5S818 went from skewed patterns in the pre-transplant sample to an equitable distribution of alleles at each locus in the 31-day post-transplant sample and then reverted back to skewed patterns in the 54-day post-transplant sample. We presume that the relative increase in the 6-repeat allele at F13A01, the 10-repeat allele at CSF1PO, and 8-repeat allele at D5S818 in the 31-day post-transplant sample is due to the elimination of many of the myelodysplastic cells as a consequence of the conditioning regimen before transplantation. Therefore, in the 31-day post-transplant sample, the recipient's normal cells, without the 6p (F13A01) and -5 (CSF1PO and D5S818) and 5q13-q31 (D5S818) deletions associated with the MDS, predominate. However, by 54 days post-transplantation, the shift in the ratio of alleles at these loci suggests that the myelodysplastic cells, with their associated chromosome 5 and 6p deletions, are assuming a greater proportion of the sample. Unfortunately, cytogenetic analysis was not performed on any post-transplant samples to confirm our speculation.

To confirm the "true" allelic STR pattern for the patient without the superimposed MDS cytogenetic changes, the molecular laboratory requested a nonhematopoietic sample from the patient. However, before a buccal swab sample could be acquired, the patient developed clinical sepsis with vancomycin-resistant enterococcus bacteremia,

candidemia, and a hospital-acquired pneumonia. She became comatose and died 3 months after her transplantation. However, we subsequently acquired some DNA from our HLA laboratory that had been isolated from peripheral blood from this patient almost 2 months before her BM transplant. In addition, the laboratory acquired several archived formalin-fixed paraffin-embedded specimens (gall bladder and skin) arising from prior surgical procedures, including one (gall bladder) that pre-dated her MDS diagnosis (Figure 1). These surgical specimens were deparaffinized, and DNA was extracted using a DNeasy Tissue kit (Qiagen) per the manufacturer's instructions. All samples were tested using CTTv and FFFL kits (Promega) as indicated by the manufacturer. The allelic patterns are presented in Figure 4, A and B. These analyses confirmed that the minor peaks that were observed in the initial pre-transplant and 54-day post-transplant samples are patient specific. All samples showed amplification of 5 and 6 repeats at the F13A01 locus and 10 and 11 repeats at the CSF1PO locus. All alleles at these loci were amplified equally in the gall bladder and HLA specimens, but in the skin biopsy, one allele at each locus (again, the 6-repeat allele at F13A01 and 11-repeat allele at CSF1PO) was reduced to 66% (F13A01) or 73% (CSF1PO) relative to that of the major allele. The skin biopsy, diagnosed as erythema multiforme, was taken 1 month before the patient's diagnosis of AML. It is likely that the same cytogenetic changes observed subse-

Figure 4. Capillary electropherograms showing amplification peaks for the F13A01 (A) and CSF1PO (B) STR loci in recipient blood-, skin-, and gall bladder-drawn samples pre-transplant and recipient 31-day and 54-day post-transplant samples. **A:** A minor peak at 6 repeats is present in the pre-transplant and 54-day post-transplant samples for the F13A01 locus, but this peak is of equal amplitude and area as the other peak in the 31-day post-transplant sample. **B:** Similarly, a diminished peak at 10 repeats is present in the pre-transplant and 54-day post-transplant samples for the CSF1PO locus, but the corresponding peak is of equal amplitude and area as the other peak in the 31-day post-transplant sample.

quently in the myeloblasts of the blood and bone marrow of the patient, and causing F13A01 and CSF1PO allelic deletions, were already evident in the skin biopsy. Curiously, the HLA specimen that was collected almost 8 weeks before her transplant and 6 weeks before receipt of the initial recipient pre-transplant sample showed equal amplification of all alleles at the F13A01 and CSF1PO loci (Figure 4, A and B). This suggests the absence of myeloblasts with the -5 and 6p deletions that were responsible for producing the skewed amplification pattern for these alleles in other specimens. A bone marrow biopsy immediately after the second induction attempt showed persistent myeloblasts (4%) and an excess of atypical megakaryocytes. We can only speculate that the clones that emerged immediately after the second induction were not those with -5 and 6p deletions that were evident later in her disease.

In conclusion, this case illustrates the need for comprehensive evaluation of pertinent clinical and laboratory data during engraftment analysis to identify potential sources for error in interpretation of STR analysis. As in this case, this would involve the potential presence of any pre-existing chromosomal abnormalities in the patient's pre-transplant sample that may affect analysis of the STR loci used in BMT engraftment monitoring. The use of alternative samples to those of hematopoietic origin, such as buccal swabs, could obviate potential problems encountered due to chromosomal changes that are part of a pre-existing hematopoietic disease process. In the cur-

rent study, we were able to make use of archived surgical pathology samples that predated the patient's MDS to evaluate this patient's "true" STR pattern. Chromosomal changes may also evolve in a patient's cells after transplantation, leading to misinterpretation of such chimerism studies. For instance, Zhou et al⁴ described a chronic myelogenous leukemia patient treated by BMT who underwent relapse and developed a near-haploid karyotype in blast crisis that resulted in loss of recipient-specific alleles and an aberrant full-donor engraftment pattern when analyzed using a single informative VNTR locus. Similarly, pre-existing or evolving chromosomal aberrations could potentially affect donor cells with erroneous results. Skekhter-Levin et al⁵ described a case of MDS involving monosomy of chromosome 7 that evolved from donor cells after BMT of a patient with acute lymphoblastic leukemia. A similar case of monosomy 7 transformation of donor cells after allogeneic BMT has been reported in a patient with severe congenital aplastic anemia.⁶ Such reports underscore the need to evaluate multiple polymorphic loci, representing different chromosomes, of the recipient and donor during bone marrow engraftment monitoring. Moreover, periodic evaluation by cytogenetic analysis and complementary methods such as XY interphase fluorescence *in situ* hybridization analysis for sex-mismatched transplants or other fluorescence *in situ* hybridization analyses, should be included to monitor residual disease and clonal evolution and further aid in STR interpretation.

References

1. Van Deerlin V, Leonard D: Bone marrow engraftment analysis after allogeneic bone marrow transplantation. *Clinics Lab Med* 2000, 20:197–225
2. Nollet, F Billiet J, Selleslag D, Criel A: Standardization of multiplex fluorescent short tandem repeat analysis for chimerism testing. *Bone Marrow Transplant* 2001, 28:511–518
3. LeClair B, Fregeau CJ, Aye MT, Fournay RM: DNA typing for bone marrow engraftment follow-up after allogeneic transplant: a comparative study of current technologies. *Bone Marrow Transplant* 1995, 16:43–55
4. Zhou M, Sheldon S, Akel N, Kileen AA: Chromosomal aneuploidy in leukemic blast crisis: a potential source of error in interpretation of bone marrow engraftment analysis by VNTR amplification. *Mol Diagn* 1999, 4:153–157
5. Skekhter-Levin S, Bloom EJ, Swerdlow SH, Sherer ME, Wald N, Gollin SM: Acquired monosomy 7 in donor cells in a patient treated for acute lymphoblastic leukemia with bone marrow transplantation. *Cancer Genet Cytogenet* 1997, 95:190–197
6. Lang Z, Dinndorf P, Ladisch S, Bayever E, Reaman G: Chromosomal transformation in donor cells following allogeneic one marrow transplantation. *Bone Marrow Transplant* 2004, 33:1253–1256