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Conventional cytogenetics and FISH evaluation of chimerism after sex-mismatched bone marrow transplantation (BMT) and donor leukocyte infusion (DLI)

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Abstract

Background and Objectives. **Sensitive and quantitative cytogenetic methods to better assess the biological significance of post-BMT chimerism have been recently developed. In this study, we compared the results of chimerism analysis and evolution employing conventional cytogenetics and fluorescence *in situ* hybridization (FISH) in 16 patients after sex-mismatched BMT, and in 5 patients after donor lymphocyte infusion (DLI) to treat post-BMT relapse.**

Design and Methods. **FISH studies were performed using separate digoxigenin labeled centromeric DNA probes for the X (pDMX1) and Y (DYZ1/DYZ3) chromosomes. To this purpose, different types of samples were used: bone marrow (BM) and peripheral blood (PB) slides processed for conventional cytogenetics, and routine BM and PB smears.**

Results. **Results of chimerism studies performed on different types of samples showed no significant differences. No significant differences in the ability to identify the sex of each cell with both pDMX1 and DYZ1/DYZ3 probes were found and the results obtained from independent experiments showed a high linear correlation. Chimerism analysis by FISH showed initial mixed chimerism after BMT in 10 patients. Seven of these patients were also studied by conventional cytogenetics and 2 of these showed mixed chimerism. Seven of the former 10 patients evolved to complete donor chimera. 6 patients showed cytogenetic or hematologic bone marrow relapse, 3 of which were preceded by mixed chimaerism as revealed by FISH studies. FISH studies permitted an easy and accurate monitorization of the response to DLI in 5 relapsed patients, showing an increase in the proportion of donor cells in 4 patients as they reached a new complete remission.**

Interpretation and Conclusions. **Both FISH and conventional cytogenetics are quantitative methods to assess chimerism. However, FISH is more sensitive, accurate and can even be applied on routine BM and PB smears. Furthermore, its combination with immunophenotyping approaches to quantify chimerism on cell subpopulations, will help to clarify post-BMT chimarism significance.**

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The persistence or reappearance of recipient cells following allogeneic bone marrow transplantation (BMT) is not uncommon, and the methodology to study the donor/recipient origin of the different hemopoietic cells, their relative presence, i.e. chimerism, and its biological significance are a matter of active research.[1] Mixed chimerism can be associated with graft rejection or disease relapse but may also coexist with clinical remission.[2] Monitoring of chimerism in BMT recipients is important for an early diagnosis of engraftment and for the optimization of the post-BMT therapy. Conventional cytogenetics[3] has limited sensitivity to assess chimerism. Recently, fluorescence *in situ* hybridization (FISH)[4] or restriction endonuclease *in situ* digestion (REISD)[5] have shown higher sensitivity and permit quantitative analysis even targeting interphase cells. Furthermore, FISH using specific probes for leukemia-related rearrangements may be used to analyze minimal residual disease.[6,7]

In this study, we compared the results of chimerism analysis and evolution employing conventional cytogenetics and FISH in 16 patients after sex-mismatched BMT, and in 5 patients after donor lymphocyte infusion (DLI) to treat post-BMT relapse.

Patients and Methods

Patients

Sixteen patients, 9 males and 7 females, received a sex-mismatched allogeneic BMT between 1993-1995 in our institution. Their clinical characteristics, diagnosis, conditioning regime, graft vs host disease (GVHD) prophylaxis and development of acute and chronic GVHD are shown in Table 1. Fifteen donors

were HLA-identical siblings and one patient (UPN 214) received an HLA-identical unrelated BMT. Five patients who relapsed after BMT, 2 AML (UPNs 164, 256), 2 CML (UPNs 108, 214) and 1 MM (UPN 231) were treated with DLI.[8]

Conventional cytogenetics

Bone marrow cytogenetics (BM^C) studies were performed by standard procedures on slides obtained pre-BMT and at various times post-BMT from 24-hour unstimulated cultures. G-Wright banding was routinely performed after overnight heating of the slides at 65°C in a dry oven. A median of 20 metaphases (range 5-25) was analyzed for each sample (Tables 3 and 4). Karyotypes were described according to the *International System for Cytogenetic Nomenclature*.[9]

FISH

FISH was performed on BM^C slides from 15 patients, as well as on bone marrow smears (BM^S) and/or peripheral blood smears (PB^S) destained slides after Wright's analysis in 14 patients (Figure 1a-d). Two different digoxigenin-labelled probes, one consisting on X chromosome specific α-satellite (pDMX1; Boehringer Mannheim; Figure 1a,b) and other consisting on α- and classical satellite sequences specific for the Y chromosome (DYZ1/DYZ3; Oncor Inc.; Figure 1c,d) were used. Hybridization conditions were those provided by the supplier.

Routine peripheral blood cytogenetics (PB^C), PB^S and BM^S slides from 20 normal donors (10 males, 10 females), as well as BM^C slides from 10 normal individuals (5 males, 5 females) were used as control samples (Table 2). PB^C slides were only used for control evaluation purposes. The number of hybridization signals per nucleus was scored in a minimum of 300 interphases from each sample.

Statistical methods

To establish the minimal proportional cut-off value for a positive control result (proportion of positive cells in males using DYZ1/DYZ3 probe and in females using pDMX1 probe), the estimates were made from the lowest proportion of each series of 10 control samples and computed from the one-sided 95% confidence interval for a binomial distribution based on the analysis of 500 cells per sample. The lower limit of this confidence interval (LLCI) was used to delimit the range of positive results in each of the 4 control samples (Table 2).[10]

To establish the superior proportional value limit for a negative control (proportion of positive cells in males using pDMX1 probe and in females using DYZ1/DYZ3 probe), the estimates took into account the highest positive proportion for each series of 10 control samples and then computed the one sided 95% confidence interval for a binomial distribution analyzing 500 cells per sample. The upper limit of such confidence interval (ULCI) was used to delimit the range of negative results (false positive) in each of the 4 control samples used (Table 2).[10]

The results obtained by FISH with both DNA probes used in this study in different control samples

Table 1. Characteristics of the sixteen sex-mismatched BMT recipients.

UPN	Age	Sex	Disease and status at BMT	Conditioning regimen	GVHD prophylaxis	aGVHD (grade)	cGVHD
108	19	M	CML/CPh	TBI+Cy	CsA+MTX	0	No
131	20	M	CML/APh	Bu+Cy	CsA	I	No
164	27	F	sAML/relapse	Bu+Vp-16	CsA	I	No
214	33	M	CML/CPh	TBI*+Cy	CsA+MTX+C	I	No
227	15	F	ALL/relapse	TBI+Cy	CsA	I	No
229	34	M	MDS	TBI+Cy	CsA+PRED	II	Yes
231	35	M	MM	TBI+Cy	CsA+MTX	I	No
234	35	F	AML-M3/1st CR	Bu+Cy	CsA+MTX	II	Yes
241	44	M	MDS	Bu+Cy	CsA+MTX	I	No
243	5	M	ALL/3rdCR	TBI+Cy	CsA	I	Yes
256	17	F	AML-M1/1st CR	TBI+Cy	CsA+MTX	II	No
257	8	F	ALL/2ndCR	TBI+Cy	CsA+MTX	II	Yes
258	18	F	AML-M2/1st CR	Bu+Cy	CsA+MTX	IV	–
267	14	M	AML-M2/1st CR	Bu+Cy	CsA+MTX	0	No
275	34	F	AA	Cy*	CsA+MTX	IV	–
280	31	M	AML-M4/1st CR	Bu+Cy	CsA+MTX	I	No

UPN: Unique patient number. CR: Complete remission. CML: Chronic myelogenous leukemia. CPh: Chronic phase. APh: accelerated phase. AML: Acute non-lymphoblastic leukemia. sAML: Secondary AML. ALL: Acute lymphoblastic leukemia. MM: Multiple myeloma. MDS: Myelodysplastic syndrome. AA: Aplastic anemia. TBI: Toyal body irradiation 12 Gy in 6 fractions. TBI: 13.5 Gy in 6 fractions. Cy: Cyclophosphamide 60 mg/kg/day x2. Cy*: Cyclophosphamide 50 mg/kg/day x4. Bu: Busulfan 4 mg/kg/day x4. Vp-16: 60 mg/kg/day. CsA: Cyclosporin A. MTX: Methotrexate. PRED: Prednisone. C: Campath 1G antibody.*

Table 2. FISH control results.

Probe	Controls n=20*		BM^C	PB^C	BM^S	PB^S
DYZ1/DYZ3	positive (males)	R	98.8-100	98.2-100	98.6-100	98.4-100
		LLCI	97.6	96.9	97.4	97.1
	negative (females)	R	0.08-0.6	0.1-0.2	0.05-0.4	0.08-0.4
		ULCI	1.54	0.94	1.25	1.25
pDMX1	positive (females)	R	96.8-99.4	96.6-98.7	97-99.2	97-98.9
		LLCI	95.2	94.9	95.4	95.4
	negative (males)	R	0-1.4	0.1	0-1.2	0-1.2
		ULCI	2.6	2.09	2.35	2.35

*BM: bone marrow. PB: peripheral blood. C: conventional cytogenetics slide. S: routine smear. R: range (%). LLCI: Lower limit of one-sided 95% confidence interval. ULCI: upper limit of one-sided 95% confidence interval. Positive controls: results in samples from normal individuals with sex concordant with the DNA probe used. Negative controls: results in samples from normal individuals with opposite sex of the DNA probe used (false positives). *n=10 for BM^C samples (see text).*

Table 3. Outcome and chimerism follow-up of 7 female recipients after BMT.

Patients n=7	Study after BMT (+mo)	Disease status	Conventional cytogenetics	FISH Y-probe (% Cels. XY)			FISH X–probe (% Cels. XX)			Chimerism status	Outcome (+mo)
			Recipient cells (XX)[a]	BM[C]	BM[S]	PB[S]	BM[C]	BM[S]	PB[S]		
UPN 164	+2	CR	NM	52	51.3	–	–	–	–	M	Died +4.5
	+3	Relapse[1] +DLI	–	–	–	–	–	–	–	–	
	+4	Relapse[1]	NM	–	–	86	–	–	19	M	
UPN 227	+3	Relapse[2]	6/20 (30%)*	67.3	67.1	–	35.2	35.3	–	M	Died +15
	+7	CR	0/14 (0%)	89	–	90	13	–	11.5	M	
	+8	CR	0/20 (0%)	98.4	98.4	99	2.1	2	2.3	C	
	+10	CR	–	99.3	–	98.6	2.2	–	–	C	
	+12	CR	–	–	–	99	–	–	2.5	M	
	+13.5	Relapse[1,2]	6/20 (30%)**	60	–	63	36	–	34	M	
UPN 234	+3	CR	1/20 (5%)	93	–	91	10.4	–	12.2	M	Alive +20
	+6	CR	0/20 (0%)	99	99.2	–	2.4	2.3	–	C	
	+7.5	CR	–	–	–	99.4	–	–	2	C	
	+8.5	CR	–	–	–	99.7	–	–	2.1	C	
	+9.5	CR	–	–	–	99.6	–	–	1.9	C	
	+10.5	CR	–	–	–	99.4	–	–	1.5	C	
	+11.5	CR	0/20 (0%)	99.2	99	–	1.5	1.3	–	C	
	+14	CR	–	–	–	99.4	–	–	1.4	C	
UPN 256	+1.5	CR	–	–	–	97	–	–	3.9	M	Died +12
	+2.5	CR	–	–	–	99	–	–	2	C	
	+3.5	CR	0/12 (0%)	99.3	99.5	–	1.9	1.7	–	C	
	+5	CR	–	–	–	98.8	–	–	1.6	C	
	+7	Relapse1	NM	9	–	10	87.3	–	86.5	M	
	+8	Relapse1+DLI	–	–	88.1	–	–	13.5	–	M	
	+9.5	CR	0/20 (0%)	98.6	–	–	1.3	–	–	C	
UPN 257	+1	CR	–	–	–	98	–	–	3.1	M	Alive +12
	+2	CR	0/20 (0%)	99.6	99.3	99.4	1	1.2	1.6	C	
	+3.5	CR	0/20 (0%)	99.2	–	–	1.4	–	–	C	
	+4.5	CR	–	–	–	99.5	–	–	1.2	C	
	+6.5	CR	0/20 (0%)	99.4	99.1	–	1.1	1.3	–	C	
UPN 258	+1	CR	–	–	–	98.2	–	–	3.2	M	Died +2
	+1.5	CR	NM	–	–	99.6	–	–	1.6	C	
UPN 275	+1	CR	–	–	–	99.2	–	–	1.4	C	Died +3
	+2	CR	0/20 (0%)	99.3	99.1	–	1.2	1.4	–	C	

UPN: Unique patient number. CR: Complete remission. Relapse[1]: Hematological relapse. Relapse[2]: Cytogenetic relapse. BM[C]: Conventional cytogenetics BM slides. BM[S]: Routine BM destained smears. PB[S]: Routine PB destained smears. NM: No metaphases. DLI: Donor leukocytes infusion. +mo: Month after BMT. M: Mixed chimerism. C: Complete chimerism.

[a]number of recipient metaphases/number of metaphases analyzed (percentage);

**Karyotype of all recipient cells: 48 XX, +X, +X, iso (7q), –8, –9, add (14q), t(2;12)(p?;q?), t(7;20)(q22;q13);*

***Karyotype of all recipient cells: 48 XX, +X, +X, add (2p), t(4;?)(q?;?), iso (7q), –8, –9, –10, +12, –13, +22q–, +mar(2)*

Table 4. Outcome and chimerism follow-up of 9 male recipients after BMT.

Patients n=7	Study after BMT (+mo)	Disease status	Conventional cytogenetics	FISH Y-probe (% Cels. XY)			FISH X–probe (% Cels. XX)			Chimerism status	Outcome (+mo)
			Recipient cells (XX)[a]	BM^C	BM^S	PB^S	BM^C	BM^S	PB^S		
UPN 108	+22	CR	0/20 (0%)	–	–	–	–	–	–	C	Alive +77
	+50	Relapse[2]	14/20 (70%)*	25	–	–	73	–	–	M	
	+59	Relapse[2]	5/14 (35.7%)*	40	39	42	57	57.2	59.3	M	
	+64	Relapse[2]+DLI	6/20 (30%)*	36	–	–	59	–	–	M	
	+65	Relapse[2]	–	–	–	20	–	–	75.4	M	
	+66	Relapse[2]	4/20 (20%)*	9.8	10.1	–	86.5	86.1	–	M	
	+68	CR	0/20 (0%)	1	–	1.2	97.3	–	98.1	C	
UPN 131	+2	CR	0/9 (0%)	2	–	–	95	–	–	M	Died +5
	+3.5	Relapse[2]	4/14 (28.6%)**	9	–	–	87	–	–	M	
UPN 214	+2	CR	0/20 (0%)	3	–	–	95	–	–	M	Died +12
	+4	CR	0/20 (0%)	–	–	–	–	–	–	C	
	+5.5	Relapse[2]	7/20 (35%)*	–	–	–	–	–	–	M	
	+11	Relapse[2]+DLI	2/20 (10%)*	36	–	–	60	–	–	M	
	+12	CR	0/25 (0%)	4	3.9	–	92	92.3	–	M	
UPN 229	+2	CR	0/20 (0%)	0.98	–	–	98	–	–	C	Died +8
	+8	CR	0/15 (0%)	1	0.9	1.2	96.7	96.3	97	C	
UPN 231	+2	CR	0/5 (0%)	0.7	–	–	98	–	–	C	Alive +23
	+8	Relapse[3]+DLI	0/20 (0%)	0.4	0.7	0.6	98.2	98.4	98.5	C	
	+12	CR	–	–	–	0.5	–	–	98.4	C	
UPN 241	+1.5	CR	0/20 (0%)	0.5	–	–	–	–	–	C	Alive +18
	+5.5	CR	0/15 (0%)	1.18	1.2	–	97.6	97.2	–	C	
	+7	CR	–	–	–	1	–	–	98	C	
	+8	CR	–	–	–	0.4	–	–	98.6	C	
	+9	CR	–	–	–	0.2	–	–	98.8	C	
UPN 243	+1	CR	0/17 (0%)	0.7	0.9	0.3	98.6	98.2	98	C	Alive +18
	+3.5	CR	–	–	–	0.7	–	–	97.9	C	
	+5	CR	NM	0.9	–	0.6	98.2	–	98.2	C	
	+6	CR	–	–	–	1	–	–	–	C	
	+7	CR	0/15 (0%)	1	1.1	0.7	97.6	97.7	98.4	C	
	+8	CR	–	–	–	0.2	–	–	98.5	C	
	+9.5	Relapse[3]	0/13 (0%)	1.2	–	–	98.5	–	–	C	
	+12	CR	–	–	–	0.9	–	–	98.3	C	
UPN 267	+1.5	CR	0/20 (0%)	1.8	1.9	2.1	98.4	98.2	–	M	Alive +8
	+3	CR	0/20 (0%)	1.6	–	1.8	97.2	–	97	M	
	+6	CR	–	–	–	1.2	–	–	98.2	C	
UPN 280	+1.5	CR	–	–	–	1.7	–	–	97	M	Alive +3
	+3	CR	0/20 (0%)	1.4	1.2	–	98.3	98	–	C	

UPN: Unique patient number. CR: Complete remission. Relapse[2]: Cytogenetic relapse. Relapse[3]: Extramedullary relapse. BM^C: Conventional cytogenetics BM slides. BM^S: Routine BM destained smears. PB^S: Routine PB destained smears. NM: No metaphases. DLI: Donor leukocytes infusion. +mo: Month after BMT. M: Mixed chimerism. C: Complete chimerism.

[a]number of recipient metaphases/number of metaphases analyzed (percentage);

**Karyotype of all recipient cells: 46 XY, t(9;22)(q34;q11);*

***Karyotype of recipient cells: 46 XY [1]/46 XY, t(9;22)(q34;q11) [3]*

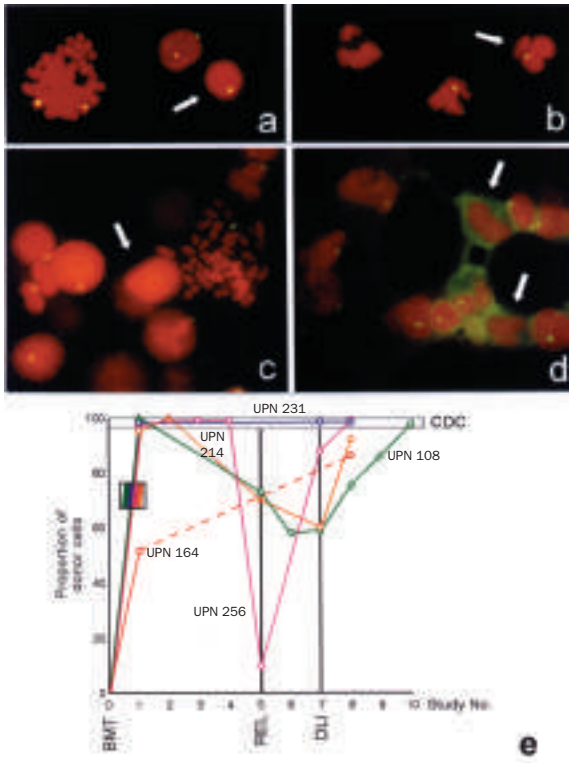

Figure 1. FISH evaluation of chimerism after sex-mismatched BMT and DLI. (a,b) FISH using the X chromosome probe pDMX1. Mixed chimerism in BM^C (a) and PB^S (b) samples in which recipient male cells (arrow) persist. (c, d) FISH using the Y chromosome specific probes DYZ1-DYZ3. Mixed chimerism in BM^C (c) and BM^S (d) samples in which recipient female cells (arrow) are observed. (e) Chimerism evolution post-BMT in the 5 patients who received DLI: UPNs 108 (green), 164 (red), 214 (orange), 231 (blue) and 256 (pink). Samples obtained at different moments after BMT (study no.) have been analyzed by conventional cytogenetics (▲) or FISH (○). CDC, complete donor chimerism. REL, relapse.

(BM^C, PB^C, BM^S and PB^S) were statistically compared using one-way analysis of variance (ANOVA). The correlation of male and female cells detected in this cohort of patients with the pDMX1 and DYZ1/DYZ3 probes were determined by the Pearson (R) correlation assay.[11] The Wilcoxon signed-rank test was used to compare the results obtained by FISH on conventional cytogenetic slides (BM^C) with those from routine smears (BM^S or PB^S).

Definition of chimerism

In conventional cytogenetic analysis, the detection of one or more recipient metaphases in the BM of a transplanted patient was defined as mixed chimerism.

A complete hemopoietic chimerism was designated from FISH studies when the proportion of opposite sex donor cells was greater than the LLCI, as established for each DNA probe in the different positive control samples, and when the proportion of recipient cells was less than the ULCI for each DNA probe obtained in the negative control samples. Mixed hemopoietic chimerism was considered to occur when the proportion of opposite sex donor cells was less than the LLCI established in the positive control samples and/or the proportion of recipient cells was greater than the ULCI established in the negative control for each DNA probe in the control samples. Donor mixed chimerism was defined when the percentage of donor-derived cells was greater than that of recipient-derived cells. Recipient mixed chimerism was defined when these proportions were reversed.

Results

Conventional cytogenetics

A total of 43 BM^C samples obtained from all 16 patients post-transplant was studied by conventional cytogenetics (Tables 3 and 4). No metaphases were observed in 5 samples (UPNs 164, 243, 256, 258).

No recipient metaphases were detected at any time post-BMT in 9 patients (UPNs 229, 231, 241, 243, 267, 270, 256, 257, 275), which therefore presented complete chimerism. Transient appearance of metaphase cells of recipient origin, i.e. mixed chimerism, occurred soon after BMT in 2 patients (UPNs 227, 234) without evidence of hematological relapse (Table 3). UPN 227 showed hematological relapse 13.5 months post-BMT (Table 3). Three more CML patients (UPNs 108, 131, 214) showed cytogenetic relapse and mixed chimerism (Table 4).

FISH

Control individuals

Normal individuals showed similar ranges of false positive and false negative results with both probes (pDMX1 and DYZ1/DYZ3) (Table 2). Furthermore, samples (BM^C, PB^C, BM^S and PB^S) were not significantly different (p: ns).

Patients

Interphase nuclei from 101 samples, 39 BM^C (Figure 1a,c), 20 BM^S (Figure 1d) and 42 PB^S (Figure 1b), obtained post-BMT from the 16 patients were analyzed by FISH (Tables 3 and 4). Within the first 3 months after BMT, mixed donor chimerism, with the percentage of recipient cells ranging between 1.7-48.7%, was observed in 10 patients (UPNs 164, 227, 234, 256, 257, 258, 131, 214, 267, 280; Tables 3 and 4). Seven of these 10 patients (UPNs 227, 234, 257, 131, 214, 267, 280) were also studied by conventional cytogenetics during the first three months post-BMT. Two of these (UPNs 227, 234) were also classified as mixed chimera by conventional cytogenetics. In the other 5 patients, the percentage of recipient cells (< 3% as revealed by interphase FISH) was too small to be detected by conventional cytogenetics. The initial mixed chimerism evolved to complete donor chimerism in 7 of these 10 patients (UPNs 227, 234,

256, 257, 258, 267, 280) in an interval of 0.5-5 months from the first study. The other 3 patients (UPNs 164, 131, 214) relapsed in 1, 1.5, and 3.5 months following the first FISH study (Tables 3 and 4). Complete chimerism in all post-BMT studies was observed using FISH and conventional cytogenetics in 5 other patients (UPNs 275, 229, 231, 241, 243). Patient UPN 108 was not analyzed by conventional cytogenetics until 22 months post-BMT and by FISH until cytogenetic relapse at 50 months showing mixed recipient chimerism.

Fourteen patients were studied by FISH using at least two types of samples (BM^C, BM^S or PB^S) (Tables 3 and 4). In 6 of them (UPNs 227, 257, 108, 231, 243, 267) the analysis was simultaneously performed on the three types of sample. No significant differences (Wilcoxon value, p: ns) were found between BM^C, BM^S and PB^S samples, either when complete chimerism, mixed chimerism or relapse was observed.

The percentage of female and male cells detected with the pDMX1 and DYZ1/DYZ3 probes in BM^C, BM^S or PB^S samples were highly linearly correlated (r=0.999). Therefore, the two different DNA probes used have a similar sensitivity for the detection of sex chromosomes in our study.

Cytogenetic and/or hematologic BM relapse was confirmed by FISH analysis in 6 patients (UPNs 164, 227, 256, 108, 131, 214) (Tables 3 and 4). One of them (UPN 227) showed a transitory relapse early after BMT, disappearing in subsequent analyses, and relapsed again 13.5 months after BMT. Mixed hemopoietic chimerism preceding the relapse was detected in 3 patients (UPNs 131, 164, 227). In 2 more patients (UPNs 231, 243), extramedullary hematological relapse was detected remaining the BM in complete chimerism.

Hematologic or cytogenetic post-BMT relapses were treated using DLI in 5 patients (UPNs 108, 164, 214, 231, 256) (Table 5). The response to this treatment was analyzed by conventional cytogenetics (Tables 3 and 4) and FISH (Table 5, Figure 1e). UPN 231 maintained complete chimerism pre- and post-DLI. UPN 108 showed a progressive change from mixed donor to complete chimerism achieving complete remission (CR). UPN 256 achieved CR and complete chimerism but died due to severe acute GVHD and infectious complications. UPN 214 reached CR with persistence of mixed donor chimerism and died from GVHD and CMV infection. Finally, UPN 164 showed a reduction of recipient cells although mixed chimerism was maintained and died in relapse due to infectious complications.

Discussion

Quantification of chimerism is far from being accurately detected by conventional cytogenetics.[3] Different methodologies, including molecular techniques, have recently improved chimerism assessment.[12] FISH analysis performed on routine smears or on slides processed for conventional cytogenetics allows a rapid and accurate quantification of the chimeric status of transplanted patient. Furthermore, the combination of FISH with immunophenotyping techniques has even allowed chimerism analysis in differ-

Pts n=5	*Diagnosis*	*Sex R/D*	*DLI (+mo)*	*MNC**	*T-Ly**	*Studies after DLI, +mo*	*Chimerism status*	*GVHD*	*DLI response*
UPN 108	CML	M/F	+64	1.6	1.0	0 +1 +2 +4	M M M C	II	CR
UPN 164	sAML	F/M	+3	6.7	3.5	-1 +1	M M	I	NR
UPN 214	CML	M/F	+11	2.1	1.4	0 +1	M M	II	CR
UPN° 231	MM	M/F	+8	3.1	1.6	0 +4	C C	II	CR
UPN 256	AML	F/M	+8	16.6	6.2	0 +1.5	M C	III	CR

Table 5. Monitoring of hemopoietic chimerism status after DLI therapy in 5 post-BMT relapsed patients.

UPN: Unique patient number. R: Recipient. D: Donor. CR: Complete remission. NR: No response. MNC: Donor mononuclear cells infused. T-Ly: Donor T lymphocytes infused. DLI, +mo: Donor leukocytes infusion, started month after BMT. DLI (+mo): month after DLI. M: Mixed chimerism. C: Complete chimerism.
**x10⁸/kg; °this patient had an extramedulary relapse, at that time a complete chimerism persisted.*

ent cell subpopulations.[11,13,14]

The results presented in this report reinforce those obtained in previous studies,[10,15] showing that FISH is more sensitive and accurate than conventional cytogenetics for the detection of mixed chimerism. In our study, FISH detected mixed chimerism in 10 out of 16 patients studied within the first 3 months after BMT, while conventional cytogenetics was useful only in 2 patients. Thereafter, 7 of these patients evolved to complete chimeras as demonstrated by conventional cytogenetics and FISH.

Dual-color FISH is superior to the single probe FISH for both X and Y chromosomes,[16] since it permits an internal quality control of hybridization success. However, the results of chimerism quantification by interphase FISH reported here were obtained using independent digoxigenin-labeled probes for both X and Y chromosomes. No significant differences were observed in the results obtained with both probes.

Particularly interesting is the possibility of performing FISH analysis on routine smears (PB, BM or other tissues). Such studies permit a rapid and quantitative chimerism assessment since large number of interphase cells can be analyzed. Eventually, the cell lines involved in chimerism may be identified to a certain degree, since cell morphology is partially preserved. It has been suggested that marrow stromal cells and the persistence of mononuclear cells of recipient origin in the peripheral blood could influence chimerism analysis.[17] However, this does not seem to be the case in our series, since no significant differences were found when BM^C/BM^S studies were compared with PB^S analysis in the same patient. Furthermore, FISH on BM^S and PB^S allows chimerism analysis early after BMT when BM cellularity is poor and conventional cytogenetics yield scarce information.

The relationship between post-BMT chimerism and the outcome of the transplant is still under debate. Several studies have shown that stable mixed chimerism post-BMT can coexist with long clinical remission either in acute leukemias[2,4,18] or in CML patients[19] without being associated with leukemic recurrence. However, patients showing post-BMT reappearance of increasing numbers of recipient cells, particularly in CML, usually have a higher probability of relapse.[2,4] In most of the reported cases,[2,4,18,20] including our series, persistence of complete donor chimerism achieved 3 months after BMT seems to be associated with long disease-free survival. The 6 patients who showed BM hematological relapse in our series were in mixed chimerism as revealed by FISH analysis at that time. Furthermore, FISH showed pre-relapse bone marrow mixed chimerism only in 3 of them. Four of the BM relapsed patients could be studied by conventional cytogenetics (UPNs 227, 108, 131, 214) and showed mixed chimerism. In UPNs 227, 108, and 214, all the recipient metaphases showed the leukemic marker chromosomes (Tables 3 and 4). One recipient metaphase from UPN 131 showed a normal karyotype (Table 4). Therefore, the reappearance of recipient cells is usually associated with the reappearance of the disease-specific markers. As has been already published,[16] a relationship exists between mixed chimerism an disease relapse. To further clarify the biological significance of chimerism, as well as its relationship with the outcome of the transplant, accurate and prospective chimerism studies targeting different cell subpopulations will be performed in the near future.

Early insight into chimerism status is of key importance to the design of therapeutic strategies in the post-BMT treatment of transplanted patients. In the case reported here, DLI therapy was the choice for the treatment of relapses after BMT.[8,21] This method can be successfully applied after the establishment of post-BMT immune tolerance and has proven to be particularly efficient for the treatment of CML in the chronic phase and several acute leukemias.[21,22] Five relapsed patients from our series (2 AML, 2 CML and 1 MM) were treated with DLI, 4 of which achieved a new CR. One patient with extramedullary relapse did not respond to DLI (UPN 231) as previously published.[23] DLI was very efficient in reducing the proportion of recipient cells, thus driving the relapsed patients towards a complete chimerism, which was achieved in 2 patients (UPN 108, 256) and maintained in 1 (UPN 231) of them.

This mode of adoptive therapy was efficient in reverting the chimeric status, from mixed at relapse towards complete after DLI, and the achievement of complete remission. This was true even in those patients where a dramatic decrease in the proportion of donor cells was observed at relapse, as occurred in UPN 256.

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JLD-M, PLI, JG: design of the study and assessment of the patients; JLD-M and JG: partially funding of the study; JLD-M, direct supervision, recruitment of patients, contacts with participants. PLI, CL-F, NP, MSdlF: laboratory work and data collection. JLD-M, PLI and IB: clinical data collection and data handling; JLD-M, JG and IB: interpretation of results. JLD-M and IB: writing of the paper; JLD-M, JG and IB: reviewing of the manuscript; all authors: final approval of the definitive version.

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Disclosures

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