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Quantification of fluorescent STR genotyping results for chimerism control after bone marrow transplantation

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1. Introduction

Engraftment of donor stem cells after allogeneic bone marrow transplantation can be genetically monitored by PCR typing of DNA polymorphisms [1]. Successful engraftment with complete chimerism and presence of the donor's genotype in the bone marrow has to be demonstrated, and the presence of the patient's alleles has to be excluded. Detection of the patient's alleles provides evidence for an incomplete chimerism or for a relapse of malignant disease. STRs have been used successfully for this type of genetic monitoring [2]. For the present study, we have developed an approach to quantify the ratio of donor chimerism using mock mixture experiments. The usefulness of our approach is demonstrated in typical cases where the donor chimerism could be monitored over periods of more than 1 year after transplantation (Tx).

2. Methods

The degree of donor–recipient chimerism was monitored by a semi-quantitative analysis of the mixed STR genotypes. DNA was extracted from bone marrow samples as well as from FACS-separated mononuclear cells, CD3+ T cells and CD3 − mononuclear cells. Furthermore, buccal swabs were collected from donor and recipient to establish their genotypes prior to transplantation. The DNA was subjected to PCR typing

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Table 1
Calculation of relative proportions for donor and recipient cells from a chimeric genotype based on STR allele peak areas (rfu)

STR genotype		Relative proportion of recipient cells	Relative proportion of donor cells
Recipient	Donor		
a,b	*c,d*	(a + b) / (a + b + c + d)	(c + d) / (a + b + c + d)
a,b	*b,c*	2a / (a + b + c)	(b + c − a) / (a + b + c)
a,b	*c,c*	(a + b) / (a + b + c)	c / (a + b + c)
a,b	*b,b*	2a / (a + b)	(b − a) / (a + b)
a,a	*b,b*	a / (a + b)	b / (a + b)
XX	*XY*	(x − y)/(x + y)	2y / (x + y)

using the Amp*Fl*STR Profiler Kit according to the manufacturer's instructions (ABI, Weiterstadt, Germany). The resulting STR fragments were separated by capillary electrophoresis with an ABI Prism 310. Results were analysed using the GeneScan software, and the genotypes were identified by comparison to allelic ladders for all loci.

To assess the relative proportion of donor and recipient cells from these STR profiles, five informative loci were selected in each case exhibiting allelic differences, e.g. homozygosity vs. heterozygosity for the same two alleles, or the presence of three or four alleles (see Table 1). The peak areas for each allele based on relative fluorescence units (rfu) were recorded from the GeneScan analysis. The total amount of cells was defined as the sum of peak areas for each STR system. The relative proportion of donor vs. recipient alleles was then calculated in percent and the mean value and standard deviation was determined across the five selected systems. For the mock mixture experiments (Table 2), genomic DNA samples from two healthy siblings (male/female) were mixed to represent donor (female) chimerism between 50% and 95%.

3. Results and discussion

Using five informative STR loci, an excellent correlation between the observed and expected donor proportions could be observed in all the mixtures even at a ratio of 5:95 (Table 2). The standard deviations of calculated mean peak areas were below 4% across all five loci demonstrating the good correlation of this approach.

For the chimerism controls in patients, blood and/or bone marrow samples as well as control blood samples (before Tx) or mouth swabs (before or after Tx) were available. Some of the patients have been treated according to an experimental protocol using non-myeloablative conditioning to use the graft-vs.-leukemia effect for elimination of residual malignant cells [3]. The semiquantitative STR genotype analysis proved to be quite helpful for prospective monitoring the treatment, as FACS-separated mononuclear cells from the recipient (CD3-positive T-cells and CD3-negative cells) could be genotyped and quantified separately (Fig. 1).

Case study: A 46-year-old male patient with a 1.5-year history of multiple myeloma after multiple chemotherapies including autologous PBSCT who was in partial remission at transplantation. He never had neutrophil granulocytes below 500/μl and thrombocytes below 20,000/μl. The patient had a myeloid donor chimerism of 72% on day +26, which

Table 2
Calculation of relative donor/recipient chimerism based on allele peak areas (rfu) from five informative STR loci

Donor/recipient ratio				50:50			30:70			15:85			5:95
Locus	STR allele	Peak area	Proportion [rfu]	(Genotype) proportion	Peak area	Proportion [rfu]	Proportion [%]	Peak area	Proportion [rfu]	Proportion [%]	Peak area	Proportion [rfu]	Proportion [%]
D3S1358	15	8,003	Recipient:	*18, 18*	9,946	Recipient:		11,113	Recipient:		14,537	Recipient:	
	18	24,561	16,558	50.8%	17,892	7,946	28.5	14,471	3,358	13.1	15,807	1,270	4.2
			Donor:	*15, 18*		Donor:			Donor:			Donor:	
			16,006	49.2%		19,892	71.5		22,226	86.9		29,074	95.8
D13S317	8	6,393	Recipient:	*11, 12*	5,534	Recipient:		6,395	Recipient:		8,315	Recipient:	
	11	7,306	15,102	54.2%	2,711	4,816	31.3	1,743	2,467	16.2	893	1,064	6.0
	12	14,189	Donor:	*8, 12*	7,639	Donor:		7,119	Donor:		8,486	Donor:	
			12,789	45.8%		11,068	69.7		12,790	83.8		16,630	94.0
VWA	14	7,474	Recipient:	*14, 15*	4,667	Recipient:		3,118	Recipient:		844	Recipient:	
	15	7,315	14,789	55.2%	4,052	8,791	32.5	2,549	5,667	22.4	936	1,780	7.1
	18	11,979	Donor:	*18, 18*	18,117	Donor:		19,595	Donor:		23,373	Donor:	
			11,979	44.8%		18,117	67.5		19,595	77.6		23,373	92.9
FGA	19	10,388	Recipient:	*19, 20*	3,663	Recipient:		1,966	Recipient:		1,176	Recipient:	
	20	8,614	19,002	54.7%	3,241	6,904	33.7	1,928	3,894	20.8	897	2,073	9.0
	23.2	7,962	Donor:	*23.2, 24*	6,780	Donor:		7,383	Donor:		10,555	Donor:	
	24	7,752	15,714	45.3%	6,807	13,587	66.3	7,477	14,860	79.2	10,527	21,082	91.0
Amelo.	Y	6,771	Recipient:	*XY*	3,731	Recipient:		2,361	Recipient:		815	Recipient:	
	X	21,425	13,542	48.0%	21,342	7,462	29.8	22,707	4,722	18.8	22,228	1,630	7.1
			Donor:	*XX*		Donor:			Donor:			Donor:	
			14,654	52.0%		17,611	70.2		20,346	81.2		21,413	92.9
Mean ratio													
Recipient (± S.D.)				52.6 ± 3.1%			31.0 ± 2.1%			18.3 ± 3.7%			6.7 ± 1.7%
Donor (± S.D.)				47.4 ± 3.1%			69.0 ± 2.1%			81.7 ± 3.7%			93.3 ± 1.7%

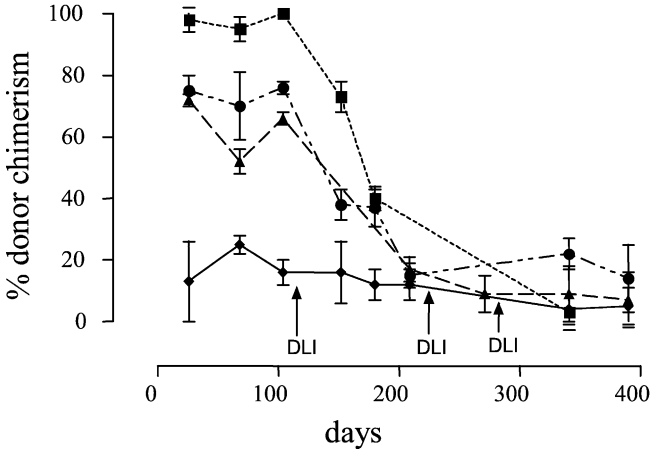

Fig. 1. Percent of genetic donor chimerism. Legend: bone marrow (- -■- -), CD3-positive (T)-cells (—◆—), CD3-negative cells (- -●- -) and whole blood mononuclear cells (- -▲- -) ± S.D.; DLI-donor lymphocyte infusion.

decreased to 9% until day +271 despite multiple donor lymphocyte infusions (0.2×10^6/kg CD34+ cells on day +112; 1×10^6/kg on day +229; 10×10^6/kg on day +277). Cytologically and immunphenotypically, he reached a partial remission (for further details, see Ref. [3]).

Thus, a STR-based semiquantitative measurement of mixed donor/recipient chimerism can be performed reliably in bone marrow transplantation monitoring. To date, we have monitored approximately 100 transplantation cases using this method.

References

[1] V.M. Van Deerlin, D.G. Leonard, Bone marrow engraftment analysis after allogeneic bone marrow transplantation, Clin. Lab. Med. 20 (2000) 197–225.

[2] J.D. Nuckols, B.K. Rasheed, R.C. McGlennen, S.H. Bigner, T.T. Stenzel, Evaluation of an automated technique for assessment of marrow engraftment after allogeneic bone marrow transplantation using a commercially available kit, Am. J. Clin. Pathol. 113 (2000) 135–140.

[3] S. Kreiter, N. Winkelmann, P.M. Schneider, M. Schuler, T. Fischer, A.J. Ullmann, C. Huber, H.G. Derigs, K. Kolbe, Failure of sustained engraftment after non-myeloablative conditioning with low-dose TBI and T-cell-reduced allogeneic peripheral stem cell transplantation, Bone Marrow Transplant. 28 (2001) 157–161.