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REVIEW

SPOTLIGHT

Chimerism and outcomes after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning

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Allogeneic hematopoietic cell transplantation (HCT) following nonmyeloablative conditioning has been extensively evaluated in patients with hematologic malignancies who are ineligible for conventional HCT because of age or medical comorbidities. Nonmyeloablative regimens have led to an initial state of mixed hematopoietic chimerism defined as coexistence of donor- and host-derived hematopoiesis. While nonmyeloablative regimens have been associated with reduced regimen-related toxicities in comparison with conventional myeloablative conditioning, graft rejection, graft-versus-host disease (GVHD), and disease progression have remained significant challenges. In this article, after briefly introducing current techniques for chimerism assessment, we describe factors affecting donor chimerism levels after nonmyeloablative conditioning, and then review data suggesting that chimerism assessment early after HCT might help identify patients at risk for graft rejection, GVHD and relapse/progression. Finally, we discuss how these observations have opened the way to further research protocols evaluating manipulation of postgrafting immunosuppression, and/or infusion of donor immune cells.
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Introduction

In Greek mythology, the 'Chimera,' as described by Homer, was a fire-breathing monster with the head of a lion, the body of a goat and the tail of a serpent, who terrorized the land of Lycia before being eventually killed by Bellerophon. Three thousand years later, the term 'chimerism' was introduced in the field of medicine by Anderson *et al.*[1] to describe 'organisms whose cells derive from two or more zygote lineages.' In hematopoietic cell transplantation (HCT), chimerism refers to the presence of lympho-hematopoietic cells of donor origin after an allogeneic HCT. For practical reasons, the term 'complete or full (donor) chimerism' has been defined as >95% of cells of donor origin, while the term 'mixed chimerism' is used to describe patients with 5–95% cells of donor origin in hematopoietic tissues[2] (Table 1).

Mixed chimerism was first described in patients with advanced acute leukemia[3] or severe aplastic anemia[4,5] conditioned with high-dose cyclophosphamide, with or without added antithymocyte globulin (ATG). In patients with aplastic anemia, mixed host/donor chimerism was associated with higher risk of graft rejection, but lower incidence of acute graft-versus-host disease (GVHD).[4,5] Stable mixed chimerism was also found in some patients with hematologic malignancies given unmodified marrow grafts after myeloablative conditioning, although its impact on HCT outcomes remained controversial.[5–8] Further, initial mixed chimerism was frequently observed among patients receiving T-cell-depleted marrows after myeloablative conditioning,[9–11] indirectly evincing that the recipients' lympho-hematopoiesis was destroyed not only by the conditioning regimens but also by donor immunocompetent cells contained in the grafts (graft-versus-host effects), even after myeloablative conditioning. Finally, mixed donor/host chimerism in T-cells, a compartment that has not been involved in the neoplastic process in chronic myeloid leukemia, was shown to be an independent marker for subsequent disease relapse in patients with chronic myeloid leukemia given T-cell-depleted marrows after myeloablative conditioning,[12] suggesting that mixed donor/host T-cell chimerism reduced graft-versus-tumor effects.

Reduced-intensity or truly nonmyeloablative conditioning regimens followed by allogeneic HCT have been increasingly used in patients with hematologic diseases who are not considered candidates for conventional HCT because of age, medical comorbidities, or prior failed myeloablative HCT, and in selected patients with solid tumors (recently reviewed in references[13–15]). Those regimens have relied mainly on the immune-mediated graft-versus-tumor effects for tumor eradication.[16–22] Criteria for nonmyeloablative conditioning as first proposed by Giralt and Champlin have included: (1) no eradication of host hematopoiesis, (2) prompt hematologic recovery (<4 weeks) without HCT and (3) presence of mixed chimerism upon engraftment.[23,24] The criteria determining whether a conditioning regimen is nonmyeloablative versus reduced in intensity have been somewhat arbitrary and controversial.[25] The distinction might be clinically meaningful given that a recent study from the MD Anderson Cancer Center demonstrated that nonmyeloablative conditioning is associated with increased risk of secondary graft rejection, decreased risk of nonrelapse mortality, and perhaps higher risk of relapse in comparison with reduced-intensity regimens.[26] In addition, while initial mixed chimerism was observed in most patients given nonmyeloablative conditioning,[27–30] many patients given grafts after reduced-intensity conditioning achieved full donor T-cell and granulocyte chimerism as early as 14 days after HCT.[31] In this review, after briefly introducing current methods for chimerism assessment, we describe factors affecting donor chimerism levels after nonmyeloablative conditioning, and then review data suggesting that chimerism assessment early after HCT might help identify patients at risk for graft rejection, GVHD, and relapse/progression. Finally, we discuss how these

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observations have opened the way to further research protocols evaluating manipulation of postgrafting immunosuppression, and/or infusion of donor immune cells in patients with low donor chimerism levels.

Current methods for chimerism analyses

Several methods have been developed to assess the level of donor engraftment after allogeneic HCT (for excellent reviews see references[1,32–34]). Early methods were based on analyses of erythrocyte antigens, leukocyte isoenzymes or conventional cytogenetics. However, these methods were time consuming, and/or had limited sensitivity and quantitative accuracy (Table 2). In the 1980s, methods based on restriction fragment length polymorphisms (RFLP) analysis were developed,[10] but were progressively abandoned, in part due to the large amounts of DNA required.[1,32,33] Currently, fluorescent *in situ* hybridization of sex chromosomes (XY-FISH) and polymerase chain reaction (PCR)-based analysis of polymorphic DNA sequences (such as variable number of tandem repeats (VNTR) or short tandem repeats (STR)) are the most widely used techniques, while real-time PCR techniques based on analysis of the Y chromosome or on single nucleotide polymorphism (SNP) are increasingly evaluated.[1,32,33]

XY-FISH

XY-FISH has remained widely used to assess degrees of donor engraftment after HCT.[35] The strengths of this technique are a high-sensitivity (<1%), and a quantitative accuracy better than what is achievable with most PCR-based assays.[32] Unfortunately, this technique can only be used in sex-mismatched HCT. Other limitations include loss of the Y chromosome observed in cells from older males, and in some tumor cells.[32]

Table 1 Definitions

Term	*Definition*
Graft rejection	<5% of T-cells of donor origin
Mixed T-cell chimerism	5–95% of T-cells of donor origin
Full (complete) donor T-cell chimerism	>95% of T-cells of donor origin
Increasing T-cell chimerism	Increasing percentage of T-cells of donor origin

VNTR- or STR-PCR

Some core DNA sequences are tandemly repeated in the genome.[36] The number of such tandem repeats varies among individuals and are inherited as codominant Mendelian traits.[32,33] Repeats can be composed of sequences of 8–100 bp in length (VNTR or 'minisatellites'), or of sequences of 2–8 bp in length (STR or 'microsatellites').[32] VNTR and STR markers have an advantage over XY-FISH in that they can be used for virtually all donor–recipient pairs.[37] In addition, when used in combination with DNA amplification by PCR, only a small number of cells (<1000) are required for the test.[38] For quantification, PCR products are electrophoresed on agarose gels,[36] hybridized with ^{32}P-labeled probes, autoradiographed and quantitated by phosphorimaging,[30] or PCR is carried out with fluorescently labeled primers and the PCR product then visualized, for example, using the ABI 310 sequencer (Applied Biosystems, Foster City, CA, USA).[37,39] Results obtained with STR or VNTR markers appear to be similar.[40] The sensitivity of STR analyses has been limited by slipped-strand mispairing of product and template strands during PCR (stutter peaks), which might induce overlap of donor and recipient alleles, and which are more frequent with smaller repeat length. Thus, depending on fragment length and efficiency of amplification, the sensitivity of VNTR- or STR- PCR ranges from 1 to 5%, but can approach 0.1% in favorable cases.[32,33] STR-PCR technique has been further improved by amplification of different loci in a single multiplex reaction,[37,38] and quantitative accuracy with multiplex PCR has approached that using XY-FISH.[38]

Real-time PCR

While XY-FISH and multiplex STR-PCR have been successful techniques for accurately assessing chimerism levels, and are currently the techniques of choice to monitor T-cell chimerism

Table 2 Methods for determining chimerism.

Assay (reference)	*Sensitivity (%)*	*Quantitative accuracy*	*Disadvantages*
Erythrocyte antigens[32]	0.1–0.5	Moderate	Studies limited to the erythroid lineage
Cytogenetics[32]	10–20	Low	Low sensitivity/accuracy Studies limited to cells in metaphase
HLA antigens[93,94]	Variable	Variable	Limited to HLA-mismatched pairs Reagents able to specifically detect many of the HLA-alleles are not yet available
FISH[32,33]	0.1–2	Very-high	Only available for sex-mismatch HCT (or when an informative autosomal marker is present). Y chromosome loss in tumor cells and with aging
RFLP[1,32]	5–20	Moderate	Technical difficulty Moderate sensitivity/quantitative accuracy High DNA requirement
VNTR/STR with phosphorimaging	1–5	Moderate	Moderate sensitivity/quantitative accuracy Radioactivity
Fluorescence-based STR – PCR[33,38]	1–5	High[a]	Moderate sensitivity
Real-time PCR[1,33,34]	0.001–1	Moderate	Moderate quantitative accuracy

FISH, fluorescent *in situ* hybridization; HCT, hematopoietic cell transplantation; HLA, Human leucocyte antigen; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphisms; STR, short tandem repeats; VNTR, as variable number of tandem repeats.
[a]When multiplex PCR approaches are used.

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after nonmyeloablative or reduced-intensity conditioning, it has been thought that a sensitivity between 1 and 5% is insufficient to detect (increasing) minimal residual disease and to predict imminent relapse after myeloablative conditioning. One approach to overcome this limitation has been to isolate the population of interest (for example CD34+ cells in patients with acute leukemia, CD138+ cells in patients with multiple myeloma, or CD19 cells in patients with chronic lymphocytic leukemia) by flow cytometry or immunomagnetic selection, and to perform XY-FISH or STR-PCR in this selected cell subpopulation.[32,41,42] This strategy may increase significantly the sensitivity of the assay, allowing pre-emptive therapy with DLI and/or targeted agents.[33,43] Another approach consists of using real-time PCR amplification of DNA polymorphisms. As only unique DNA sequences are useful for real-time PCR, repetitive DNA polymorphisms like VNTR or STR cannot be used. Thus, several other markers have been investigated, including Y chromosome markers, single nucleotide polymorphisms (SNP), and short insertion/deletion polymorphisms.[44–46] The sensitivity of these techniques ranges from 0.0001 to 0.1%, probably allowing minimal residual disease quantification in patients given myeloablative conditioning or in patients given nonmyeloablative conditioning after they achieve 100% donor T-cell chimerism. However, despite recent improvements, the quantitative accuracy of real-time PCR has remained below what is achieved with XY-FISH or multiplex PCR.[33]

Kinetics of donor blood cell subsets engraftment

While nonmyeloablative conditioning usually leads to an initial state of mixed chimerism, only a few reports have analyzed the engraftment kinetics of specific hematopoietic lineages after nonmyeloablative conditioning. Childs *et al.*[27] studied chimerism evolution among various blood cell subsets in 15 patients given allogeneic HCT after conditioning with fludarabine (125 mg/m^2) and cyclophosphamide (120 mg/kg). Postgrafting immunosuppression consisted of cyclosporine (CSP) alone. The patterns of engraftment varied among their patients, but most often full donor chimerism was achieved earlier in T cells (CD3+, median time 30 (range, 14–164) days) than in myeloid cells (CD14/15+). Chimerism levels among blood myeloid cells correlated with those observed among marrow progenitor cells (CD34+, $r=0.99$), and erythroid (CD71+, $r=0.98$) cells. Similarly, chimerism levels among blood T-cells closely correlated with those observed among blood NK cells (defined as CD2+CD3- cells, $r=0.98$). In contrast, B-cell (CD19+ cells) recovery was distinct from both myeloid ($r=0.26$) and T-cell lineages ($r=0.24$).

We analyzed the kinetics of donor engraftment in peripheral blood hematopoietic subpopulations from 120 patients conditioned with 2 Gy TBI with or without fludarabine (90 mg/m^2), and postgrafting immunosuppression with mycophenolate mofetil (MMF) and CSP.[30] While patients rapidly developed high degrees of donor engraftment, most remained mixed donor/host chimeras for up to 3–6 months after HCT. Correlations between donor T-cell content and those of granulocytes ($r=0.37$), NK cells ($r=0.66$) and monocytes ($r=0.56$) were relatively weak.

Factors affecting chimerism levels after reduced-intensity or nonmyeloablative conditioning

Several factors have been associated with kinetics of donor engraftment after nonmyeloablative or reduced-intensity conditioning. These factors influence either the recipients' immune competence (thereby affecting host-versus-graft (rejection) reactions), the immune competence of the cells in the graft (thereby affecting graft-versus-host reactions), or both.

Factors affecting host-versus-graft reactions

Conditioning regimen. Not surprisingly, the intensity of the conditioning regimen has affected the speed of donor engraftment. In a preclinical canine model of nonmyeloablative transplantation, decreasing doses of TBI (from 2 to 0.5 Gy) are associated with lower donor chimerism levels.[47,48] In humans, the addition of fludarabine (90 mg/m^2) or autologous HCT (tandem autologous-allogeneic HCT) to a 2 Gy TBI conditioning is associated with higher donor T-cell chimerism levels early after HCT ($P=0.001$).[49] Further, comparing chimerism levels in patients given peripheral blood stem cells (PBSC) from related donors as treatment for metastatic solid tumors after different conditioning regimens, median day-28 donor T cell and myeloid chimerism levels were 100 (range, 100–100)% and 100 (range, 100–100)% in patients conditioned with fludarabine (125–150 mg/m^2) and melphalan (140 mg/m^2),[31] versus 92 (range, 55–100)% and 38 (range, 5–97)% in patients conditioned with fludarabine (125 mg/m^2) and cyclophosphamide (120 mg/kg),[29] versus 71 (range, 50–90)% and 91 (range, 60–99)% in patients conditioned with fludarabine (90 mg/m^2) and 2 Gy TBI,[50] respectively.

Previous chemotherapy. Valcarcel *et al.* analyzed data from 68 patients transplanted after conditioning with fludarabine (150 mg/m^2) and busulfan (10 mg/kg) (patients with myeloid malignancies, $n=28$), or with fludarabine (150 mg/m^2) and melphalan (140 mg/m^2) (patients with lymphoid malignancies, $n=40$).[51] In multivariate analysis, having received more than two lines of chemotherapy pretransplant was the only factor significantly associated with achievement of complete donor chimerism among unfractionated nucleated peripheral blood cells at day 30 after HCT ($P=0.02$). Similarly, Carvallo et *al.*,[29] analyzing data from 36 patients with solid tumors given allogeneic grafts after fludarabine (125 mg/m^2) and cyclophosphamide (120 mg/kg), found that T cell ($P=0.008$) and granulocyte ($P<0.0001$) donor chimerism levels were higher in patients previously given myelosuppressive chemotherapy in comparison to patients who were not given chemotherapy. Consistent with these observations, myelosuppressive chemotherapy received before HCT was associated with higher donor T cell ($P=0.002$), granulocyte ($P=0.002$), monocyte ($P=0.01$) and NK ($P=0.10$) chimerism levels in a study analyzing data from 120 patients given grafts from related or unrelated donors after 2 Gy TBI with or without fludarabine (90 mg/m^2).[30]

Underlying disease category. Lower levels of donor chimerism and higher incidences of graft rejection have been observed in patients with chronic myeloid leukemia or myelodysplastic syndrome, in comparison to patients with other hematologic malignancies.[18,52–56] Whether this is due to the fact that most of these patients were not given myelosuppressive chemotherapy before the HCT procedure, or whether the reason was the underlying diseases themselves (e.g., by inhibitory effects of myelodysplastic cells on donor hematopoiesis[57]) remains unclear. For example, patients with chronic myeloid leukemia or myelodysplastic syndrome were found to have lower levels of donor T-cell chimerism than patients with acute myeloid leukemia or with lymphoid malignancies ($P=0.03$) in a

study analyzing engraftment kinetics after 2 Gy TBI with or without fludarabine ($n=120$).[30] However, after adjusting for prior myelosuppressive chemotherapy or not, the impact of disease category on donor T-cell chimerism levels was no longer significant.

Factors affecting graft-versus-host reactions

Stem cell source. Maris *et al.*[53] analyzed outcomes of patients given unrelated marrow versus unrelated PBSC after fludarabine and 2 Gy TBI. Marrow recipients had lower levels of donor T-cell chimerism the first 2 months after HCT ($P<0.02$), higher incidence of graft rejection ($P=0.007$), and worse progression-free survival ($P=0.006$). In contrast with these observations, full donor chimerism was achieved rapidly in many patients given marrows after more intense (but still 'reduced intensity') conditioning regimens.[56,58]

PBSC composition. The first study analyzing the impact of PBSC composition on donor chimerism levels was reported by Carvallo *et al.*[29] The authors showed that high levels of CD34+ progenitor cells in the grafts were associated with higher levels of donor myeloid chimerism early after HCT. However, no significant correlations between graft composition and donor T-cell chimerism levels were observed. We analyzed the impact of graft composition on HCT outcomes in 125 patients given PBSC from HLA-identical siblings after 2 Gy TBI with or without fludarabine.[49] Higher number of NK cells transplanted was associated with higher levels of day-28 donor T-cell chimerism ($P=0.03$). Cao *et al.*, using the same preparative regimen combining 2 Gy TBI with or without fludarabine, showed that higher numbers of transplanted CD8+ T-cells correlated with increased donor T-cell chimerism levels on day 28 after HCT ($P=0.009$), and greater likelihood for achievement of full donor T-cell chimerism ($P=0.03$) in a study analyzing combined observations in 63 patients given PBSC from either related ($n=38$) or unrelated ($n=25$) donors.[59] Finally, we analyzed the impact of graft composition on HCT outcomes among 116 patients receiving PBSC from HLA-matched unrelated donors after conditioning with fludarabine and 2 Gy TBI.[60] High numbers of donor T-cells ($P=0.03$), CD4+ T-cells ($P=0.007$), CD8+ T-cells ($P=0.29$), NK cells ($P=0.04$) and CD34+ progenitor cells ($P=0.01$) in the graft were each associated with high levels of day-28 donor T-cell chimerism. Furthermore, high numbers of CD34+ cells in the graft were associated with faster achievement of full donor T-cell chimerism ($P=0.02$). Taken together, these data suggest that higher numbers of each CD34+ progenitor cells and immune (T-cells and NK cells) cells in the grafts increase donor chimerism levels after nonmyeloablative conditioning.

Factors affecting host-versus-graft and graft-versus-host reactions

Postgrafting immunosuppression. Maris *et al.*[61] compared day-28 donor T-cell chimerism levels among patients receiving HLA-matched unrelated PBSC after nonmyeloablative conditioning with 2 Gy TBI and fludarabine, and given CSP plus MMF 15 mg/kg twice a day (bid group, $n=71$) versus MMF 15 mg/kg thrice a day (tid group, $n=103$) as postgrafting immunosuppression. Median donor T-cell chimerism levels on day 28 after HCT were 75% in the bid group, versus 92% in the tid group ($P=0.02$). In agreement with these findings, Giaccone *et al.*[62] observed a positive correlation between mycophenolic acid (the active metabolite of MMF) concentration steady-state levels on days 7 and 21 after HCT, and donor T-cell chimerism levels 28–84 days after HCT ($P=0.04$), in patients given unrelated grafts after fludarabine and 2 Gy TBI, and postgrafting immunosuppression with MMF and CSP.

Associations between engraftment kinetics and HCT outcomes

Graft rejection

Nonfatal graft rejection has remained a significant complication of HCT following nonmyeloablative or reduced-intensity conditioning in patients with chronic myeloid leukemia or myelodysplastic syndrome receiving grafts from unrelated donors.[54,56] This has prompted several groups of investigators to look whether donor chimerism levels early after HCT could predict graft rejection.

Bornhauser *et al.*[52] first observed that patients with NK-cell donor chimerism levels below 75% on days 10–30 after unrelated HCT following conditioning with fludarabine (150 mg/m^2) intraveneous (i.v.) busulfan (6.6 mg/kg), and ATG were more likely to have graft failures (three of three patients) than those with more than 75% (one of seven patients, $P=0.03$). Data from Matthes-Martin *et al.*[63] confirmed this observation by showing graft rejection in nine of 15 children (given various reduced-intensity conditioning) with <100% donor NK-cells on day 28 after HCT versus 0 of 24 children with 100% donor NK cell chimerism on the same day ($P<0.0001$).

We recently observed that day-14 T-cell and NK cell donor chimerism levels below 50% were each associated with increased risks of subsequent graft rejection ($P=0.0007$ and $P=0.003$, respectively) in a study analyzing data from 157 patients given grafts after 2 Gy TBI with or without fludarabine (Baron F *et al.*, *Biol Blood Marrow Transplant* 2005, **11**(Suppl): 31 (abstract)). Specifically, 50% of patients with donor T-cell chimerism levels below 25%, 8% of patients with donor T-cell chimerism levels between 26 and 50%, 4% of those with levels between 51 and 75%, and 0% of those with levels above 75% rejected their graft. For NK cell chimerism levels, corresponding figures were 44, 20, 0 and 4%, respectively (Figure 1a). Figure 1b shows the cumulative incidence of graft rejection according to day-28 donor T-cell chimerism levels in a group of 21 patients given unrelated grafts after nonmyeloablative conditioning as treatment for chronic myeloid leukemia.

Graft-versus-host disease

The intensity of the preparative regimens has been shown to contribute to acute GVHD physiopathology, presumably by inducing tissue damage and the release of a 'cytokine storm'.[64,65] Further, mixed donor/host hematopoietic chimerism has been associated with decreased risk of GVHD.[5,30,43,66,67] Thus, one might expect relatively decreased incidence of GVHD after nonmyeloablative versus myeloablative conditioning. Indeed several reports have shown lower incidence of acute GVHD after nonmyeloablative versus myeloablative conditioning,[68–72] including one study analyzing age-matched patients treated in a single institution.[68] However, although relatively less frequent, acute GVHD has remained a frequent complication of nonmyeloablative HCT, and has been associated with increased nonrelapse mortality and decreased progression-free survival.[19,73]

Several groups of investigators analyzed the relationship between engraftment kinetics and acute GVHD. Childs *et al.*[27] observed that achievement of full donor T-cell chimerism

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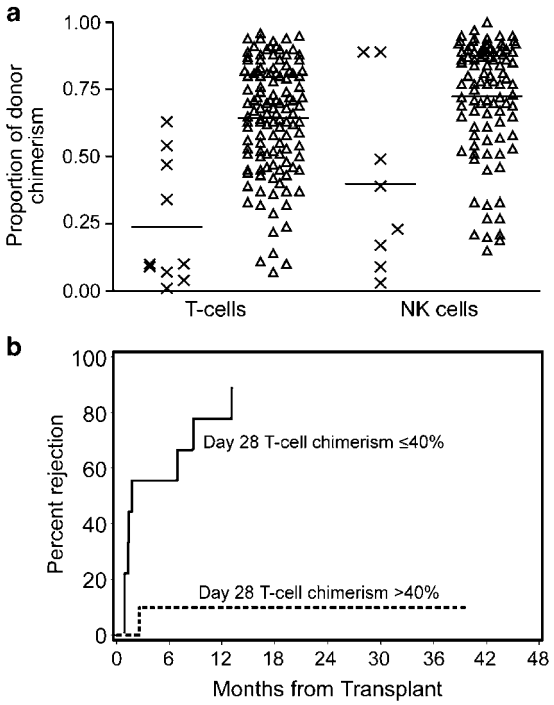

Figure 1 (**a**) Day-14 donor T-cell and NK-cell chimerism levels in patients with or without subsequent graft rejection. All patients were given grafts after 2 Gy total body irradiation with or without fludarabine. Triangle indicates sustained engraftment; × indicates graft rejection (Baron F *et al.*, *Biol Blood Marrow Transplant* 2005; **11**(Suppl): 31 (abstract)). (**b**) Cumulative incidence of graft rejection according to day-28 T-cell chimerism levels in patients with chronic myeloid leukemia given grafts from unrelated donors after 2 Gy TBI plus fludarabine (90 mg/m^2).[54]

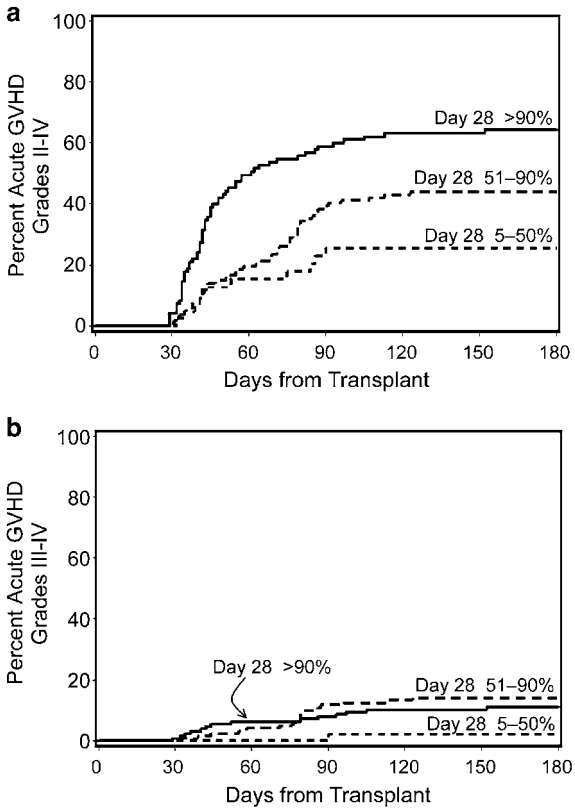

Figure 2 (**a**) Cumulative incidence of grade II–IV acute GVHD based on day-28 donor T-cell chimerism levels in patients given grafts after 2 Gy TBI with or without fludarabine ($P<0.0001$).[19] (**b**) Cumulative incidence of grade III–IV acute GVHD based on day-28 donor T-cell chimerism levels ($P=0.05$).[19]

usually preceded grade II–IV acute GVHD in patients conditioned with fludarabine plus cyclophosphamide. In contrast, Mattsson *et al.*[74] found that 82% of their patients (conditioned with four nonmyeloablative regimens, including fludarabine, busulfan, TBI, cyclophosphamide and ATG) had mixed donor/host T-cell chimerism at the time of onset of acute GVHD. In agreement with Mattsson's findings, we found that 83% of patients were mixed donor/host T-cell chimera at the time of onset of grade II–IV GVHD in a study analyzing data from 120 patients given related or unrelated grafts after 2 Gy TBI with or without added fludarabine.[30] These apparent discrepancies were probably related to the different conditioning regimens used from one study to another.

Another question has been whether or not assessment of donor chimerism levels early after HCT helped identify patients at high risk for acute GVHD. McSweeney *et al.*[18] analyzed data from 45 patients given related PBSC after 2 Gy TBI and found that patients with higher levels of donor T-cell chimerism on day 28 after HCT were at higher risk for grade II–IV acute GVHD than patients with lower levels of donor T-cell chimerism, when chimerism was analyzed as a continuous linear variable ($P=0.03$). Similar observations were made by Maris *et al.*[53] in patients given unrelated grafts after 2 Gy TBI and fludarabine. In agreement with these observations, Perez-Simon *et al.*[75] found a trend toward a lower incidence of acute GVHD in patients with mixed donor T-cell chimerism (17%) compared to patients with full donor T-cell chimerism (50%, $P=0.1$) early (days 28 to 56) after conditioning with fludarabine plus melphalan or fludarabine plus busulfan.

More recently, we analyzed the relationship between chimerism levels on day 14 after HCT among various blood subsets and acute GVHD in 157 patients given related or unrelated grafts after 2 Gy TBI with or without added fludarabine (Baron F *et al.*, *Biol Blood Marrow Transplant* 2005, **11**(Suppl): 31 (abstract)). High donor T-cell chimerism levels on day 14 ($P=0.02$) were associated with increased risks of grades II–IV acute GVHD. Specifically, among patients alive without GVHD on day 14, 38% of those with donor T-cell chimerism levels below 50%, 49% of those with levels between 51 and 75%, and 71% of those with levels above 75% had subsequent grade II–IV acute GVHD.

Taken together, these observations suggested that patients at higher risk of acute GVHD might be identified early after nonmyeloablative HCT. However, it should be emphasized, as shown in Figure 2, that cell subset chimerism analyses have been less successful in predicting patients at high risk for grade III–IV acute GVHD than in predicting patients at high risk for grade II–IV acute GVHD.

Tumor eradication

It has remained controversial whether or not achievement of full donor T-cell chimerism is mandatory to achieve disease response in patients given nonmyeloablative conditioning. Childs *et al.*[27] observed that full donor T-cell chimerism generally preceded achievement of disease responses in patients given grafts after fludarabine plus cyclophosphamide.

In contrast, Mattsson *et al.*[74] found that 40% of their patients ($n=30$) had mixed donor/host T-cell chimerism at the time of disease responses. We found comparable findings in 120 patients given related or unrelated grafts after 2 Gy TBI with or without fludarabine: 19 of 41 (46%) patients who achieved complete remissions after HCT were mixed donor/host T-cell chimera when complete remissions were achieved.[30] However, when donor T-cell chimerism levels were modeled as a continuous linear variable, high levels of donor T-cell chimerism on day 28 after HCT were suggestively associated with higher probability of achieving complete remissions ($P=0.16$).

Two reports looked at the association between donor chimerism levels and achievement of molecular remissions in patients receiving nonmyeloablative conditioning as treatment for chronic myeloid leukemia.[55,76] Uzunel *et al.* analyzed 15 patients given grafts after fludarabine (180 mg/m^2), busulfan (8 mg/kg) and ATG. Full donor T-cell chimerism was usually seen before ($n=5$; 24–90 (median 40) days), or at the time ($n=4$) of molecular remission.[76] However, one patient had molecular remission with mixed T-cell chimerism. The authors concluded that complete T-cell chimerism was probably not required to achieve molecular remissions, given the short interval between conversion to full donor T-cell chimerism and molecular negativity. We analyzed data from 13 patients with chronic myeloid leukemia who achieved molecular remission after conditioning with 2 Gy TBI with or without fludarabine.[55] Eleven of them achieved molecular remission at the time ($n=1$), or after ($n=10$) conversion to full donor T-cell chimerism, while two patients had molecular remissions 3 and 9 months before conversion to full donor T-cell chimerism, respectively.

Relapse, nonrelapse mortality and survival

Assessment of donor chimerism levels might help identify patients at higher risk of relapse after nonmyeloablative/reduced-intensity conditioning. First, high donor chimerism levels among immune cells (T-cells and NK cells) might be a surrogate for high graft-versus-tumor effects. Secondly, chimerism analyses might be useful to detect and quantitate minimal residual disease after HCT.

Chimerism among immune cells. Keil *et al.*[77] found that patients with $<90\%$ donor T-cell chimerism on day 28 after nonmyeloablative conditioning were at higher risk of relapse (55% versus 18%, $P=0.04$), and had a lower 2-year probability of progression-free survival ($P=0.002$) than patients with $\geqslant 90\%$ donor T-cell chimerism, in a study analyzing data from 38 patients given grafts after 2 Gy TBI and fludarabine (90 mg/m^2). No correlation between donor granulocyte chimerism levels and outcomes was observed. We prospectively investigated the relationship between kinetics of donor engraftment of T-cells and NK cells and outcomes in 229 patients (130 with HLA-matched related donors and 99 with unrelated donors) conditioned with 2 Gy TBI with or without fludarabine (Baron F *et al.*, 2005, *Blood*; **106** (Part 1): 119a (abstract)). High donor T-cell ($P=0.008$) and NK cell ($P=0.005$) chimerism levels on days 14–42 were both associated with decreased risk of relapse in time-dependent analyses adjusted for disease risk, while high levels of donor NK cell (but not T-cell) chimerism were associated with better overall ($P=0.003$) and progression-free ($P=0.003$) survivals. If confirmed in a larger number of patients, this observation might lead to the development of protocols evaluating infusion of donor NK cells[78] in patients with low donor NK cell chimerism levels early after HCT.

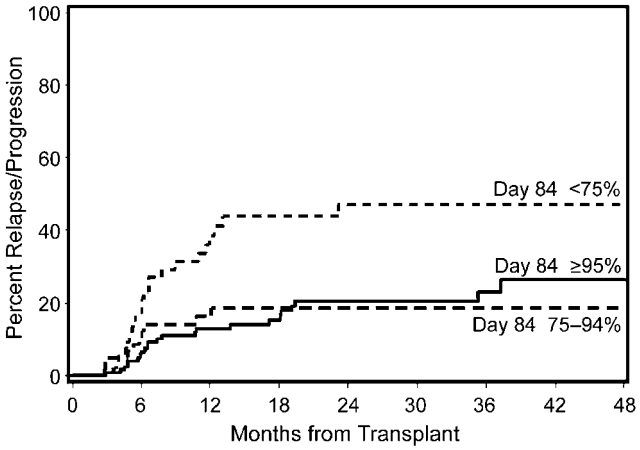

Figure 3 Cumulative incidence of relapse according to day-84 donor T-cell chimerism levels in patients reported in ref.[19] given grafts after 2 Gy TBI with or without fludarabine ($P=0.002$).

Another important clinical question has been whether or not it is necessary to achieve full donor T-cell chimerism in patients without measurable disease after nonmyeloablative or reduced-intensity conditioning to prevent disease relapse.[79] Perez-Simon *et al.*[75] found a trend for a higher relapse risk in patients with mixed donor/host T-cell chimerism as compared to patients with complete donor chimerism after conditioning with fludarabine/melphalan or fludarabine/busulfan. We investigated the impact of achievement of full donor T-cell chimerism on HCT outcome in 322 patients given related or unrelated grafts after nonmyeloablative conditioning.[19] In multivariate analyses adjusting for disease risk, comorbidity and tandem autologous/allogeneic HCT, achievement of full donor T-cell chimerism was associated with a significantly lower risk of relapse (HR = 0.5, $P=0.002$), and a suggestion for a better progression-free survival. Further, patients with donor T-cell chimerism levels $<75\%$ at day 84 were found to have higher risk of relapse (HR = 0.4, $P=0.002$) (Figure 3) than patients with $\geqslant 75\%$ donor T-cell chimerism levels. Comparable results were recently reported by Mohty *et al.*, who observed higher risk of relapse ($P=0.002$) and worse progression-free survival ($P=0.06$) in patients who had mixed donor/host T-cell chimerism in comparison to patients who had full donor T-cell chimerism on day 90 after reduced-intensity conditioning HCT as treatment for myeloid malignancies (Mohty M *et al.*, *Biol Blood Marrow Transplant* 2006; **12** (Suppl 1): 33 (abstract)).

Chimerism as a marker for minimal residual disease. In patients receiving truly nonmyeloablative conditioning, chimerism among total blood cells or among myeloid cells in patients with myeloid malignancies has been a poor marker for minimal residual disease, and has not been shown to predict HCT outcomes. For example, Niederwieser *et al.*[80] analyzed data from 19 patients with chronic myeloid leukemia given grafts after 2 Gy TBI with or without fludarabine. Slow versus rapid reduction of BCR/ABL transcripts after HCT was associated with increased risk of relapse ($P=0.006$), while low donor granulocyte chimerism levels were not. Similarly, we failed to find any association between donor granulocyte chimerism levels and disease relapse in a study analyzing data from 120 patients given nonmyeloablative conditioning.[30] Similarly, donor chimerism levels among total marrow cells have been a poor predictor of HCT outcomes after nonmyeloablative conditioning,[18] although

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one study suggested higher risk of relapse in patients with mixed donor/host marrow chimerism (57%) than in those with full donor marrow chimerism (30%, $P=0.06$) on day 30 after reduced intensity conditioning.[56] Nevertheless, serial chimerism assessments in blood or marrow sub-populations enriched for the malignant cell type (for example CD34+ cells in patients with leukemia,[41] CD19+ cells in patients with chronic lymphocytic leukemia,[43] or CD138+ cells in patients with multiple myeloma[42]) might be able to better predict relapse/progression after nonmyeloablative conditioning.

Disease-specific engraftment kinetics in nonmalignant diseases

In patients with nonmalignant diseases, low levels of donor chimerism among total blood or marrow cells, and particularly among T- and NK-cells, have been associated with increased risk of graft rejection, as observed in patients transplanted for malignant disease (nicely reviewed by Bader *et al.*[1]). In addition, assessment of disease-specific donor chimerism levels among lymphocyte subsets or erythroid cells provided valuable information that could affect clinical decisions in patients transplanted for severe combined immunodeficiency (SCID)[81] or sickle cell disease, respectively.[82]

Van Leeuwen *et al.*[81] studied engraftment kinetics in 14 children with SCID given marrows after either no conditioning, reduced-intensity conditioning, or a myeloablative regimen, and surviving more than 1 year after HCT. While peripheral blood T-cells were exclusively or predominantly of donor origin in all children, B-, NK- and myeloid- cells were either of recipient, donor or mixed origin. Interestingly, engraftment of donor NK cells was associated with increased *in vitro* NK cell function in children with defective NK cells before HCT.

Burroughs *et al.* studied data from 14 patients with SCID ($n=3$) or other immunodeficiency disorders ($n=11$) given grafts from either related ($n=7$) or unrelated ($n=7$) donors (Burroughs *et al., Blood* 2005; **106**: 134a (abstract)). Two patients had no conditioning, while 12 were conditioned with 2 Gy TBI with or without added fludarabine. Mixed or full donor chimerism among T-cell and granulocytes was observed in 10 patients, whereas four patients received a second graft because of T-cell graft rejection ($n=1$), low donor T-cell chimerism levels ($n=2$) or loss of granulocyte donor chimerism ($n=1$).

Wu *et al.*[82] analyzed donor chimerism levels among peripheral blood mononuclear cells and erythrocyte-lineage cells in four patients with sickle cell disease given allogeneic grafts after reduced-intensity conditioning. Erythrocyte-lineage chimerism was determined by β-globin RNA pyrosequencing. Interestingly, all four patients had predominant to full replacement of peripheral-blood erythrocytes with donor cells, and significant clinical improvement, despite peripheral blood monocyte cell donor chimerism levels ranging from 25 to 85% (median 50%).

Manipulation of immunosuppression and/or infusion of donor cells according to donor chimerism levels

Given the accumulating data suggesting that patients at high risk for graft rejection and relapse might be identified early after HCT by T-cell and NK-cell chimerism assessment, research protocols aimed at preventing those complications in patients with low levels of donor chimerism have been developed.

Prevention of graft rejection after nonmyeloablative conditioning

We reported data from 53 patients given DLI (median dose of 1×10^7 T-cells/kg) as treatment of persistent/progressive disease or to increase donor chimerism after HCT following conditioning with 2 Gy TBI with or without fludarabine.[83] Following DLI, grades II, III and IV acute GVHD were seen in six, two and one patient, respectively, while 10 patients developed extensive chronic GVHD. Three of 16 patients who received DLI for low or failing donor chimerism achieved full donor T-cell chimerism, four remained stable mixed chimera, and nine (including eight of 12 patients with donor T-cell chimerism level $<50\%$ at the time of DLI) eventually rejected their grafts (Figure 4a).

Therefore, we developed a research protocol evaluating safety and efficacy of the administration of pentostatin (given at 4 mg/m^2 to decrease host-versus-graft reactions) followed 2 days later by DLI (1×10^7 T-cells/kg) to reverse pending graft rejection. The results of the first 10 patients treated have been summarized recently (Sandmaier BM, *et al., Blood* 2004; **104**(Part 1): 57a (abstract)). Patients were given pentostatin 54–339 (median 91) days after HCT. Median donor T-cell chimerism level before pentostatin and DLI was 29.5 (range 5–38)%. Six of 10 patients showed increases of donor T-cell chimerism levels ranging from 63 to 100% (one example is shown in Figure 4b). In the remaining four patients, donor T-cell chimerism remained at 5–26% following DLI (none experienced graft rejection, but two received a second HCT for low donor T-cell chimerism levels and aplasia). Interestingly, five of six responding patients versus 0 of four nonresponding patients experienced grade II–IV acute GVHD ($P=0.047$). These preliminary observations suggested that immunosuppression with pentostatin followed by DLI might be more effective for conversion to full donor chimerism than DLI alone in patients with low T-cell and/or NK cell chimerism levels after HCT with nonmyeloablative conditioning.

Prevention of relapse after nonmyeloablative or reduced-intensity conditioning

Based on the observations that full donor T-cell chimerism was associated with increased graft-versus-tumor effects,[12,19] several groups of investigators evaluated the ability of DLI to convert mixed to full donor T-cell chimerism. Dey *et al.*[28] analyzed efficacy of prophylactic DLI (1×10^7 T-cells/kg) given 5 weeks after HCT, in 16 patients given marrows from HLA-identical related donors after conditioning with cyclophosphamide (150–200 mg/kg), ATG and thymic irradiation. By day 100 after HCT, 10 patients had converted to full donor T-cell chimerism, two had stable or increased donor chimerism levels, and four had rejected their marrows (including four of four patients with donor T-cell chimerism levels $<25\%$ before DLI). DLI were complicated by grade III or grade IV acute GVHD in one and four patients, respectively.

Peggs *et al.*[84] analyzed the efficacy of dose-escalating DLI given in 46 patients as treatment of persistent/progressive disease or to convert mixed donor chimerism to full donor T-cell chimerism in order to prevent relapse/progression. The conditioning regimen used before HCT consisted of fludarabine (150 mg/m^2), melphalan (140 mg/m^2), and alemtuzumab (100 mg),[84] and DLI were given at a starting dose of 1×10^6 T-cells/kg. Increasing doses were given at 3-month intervals (3×10^6, 1×10^7, 3×10^7 and 1×10^8 T-cells/kg) in the absence of GVHD if mixed chimerism or underlying malignancy persisted. Grades II–IV GVHD occurred in five of 32 (16%) (including three grade IV) sibling and seven of 14 (50%) (including four grade IV) unrelated donor recipients ($P=0.002$).

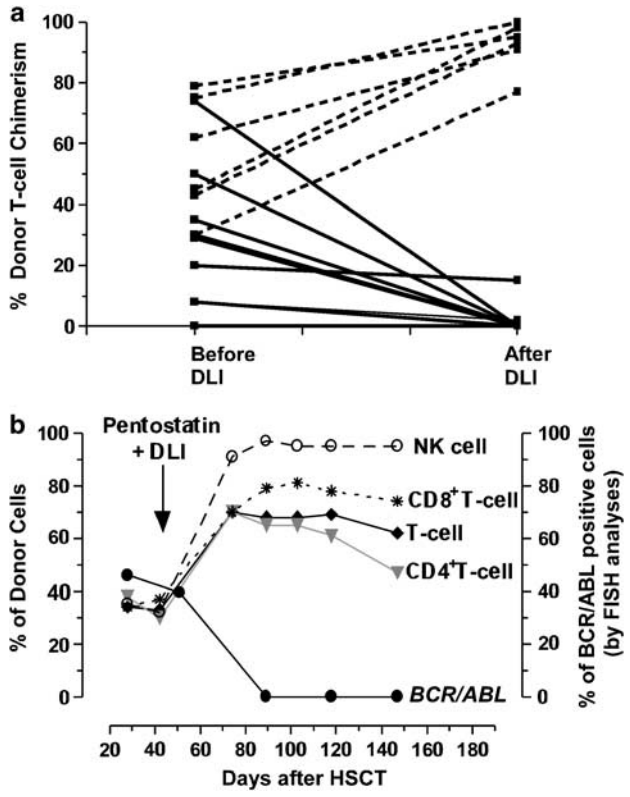

Figure 4 (**a**) Evolution of donor T-cell chimerism levels in patients given DLI for low or decreasing chimerism donor T-cell chimerism levels after nonmyeloablative conditioning.[83] (**b**) Peripheral blood donor T-cell, $CD4^+$ T-cell, $CD8^+$ T-cell and NK-cell chimerism levels, and BCR/ABL bone marrow positive cells (assessed by FISH), in a patient with chronic myeloid leukemia in first chronic phase given unrelated PBSC after 2 Gy TBI and fludarabine. The patient had low T-cell and NK-cell chimerism levels early after HCT, predicting high risk of subsequent graft rejection. The patient received pentostatin (4 mg/m^2) on day 43 followed by donor lymphocyte infusion 2 days later (Sandmaier BM *et al.*, *Blood* 2004; **104**(Part 1): 57a (abstract)). This resulted in significant increase in donor chimerism level among all subpopulations, and the patient is currently surviving in molecular remission with sustained graft >300 days after HCT.[54] Figure 4b was reprinted from *Molecular Therapy*, vol 13, Baron F, Storb R. Allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning as treatment for hematologic malignancies and inherited blood disorders (Review), pp 26–41, Copyright 2006, with permission from Elsevier.

Among 14 patients given DLI for mixed donor/host T-cell chimerism, 12 were evaluable for chimerism responses, and 10 of 12 achieved full donor T-cell chimerism.

Chimerism assessment for patients with disease relapse

Chimerism assessment has also been useful in patients with relapsed hematological malignancies after nonmyeloablative HCT. Chimerism analyses included both flow-sorted malignant cells, to determine whether the disease originated in cells of donor[85,86] or host origin, and T-cells, to rule out concomitant graft rejection. Donor lymphocyte infusions, with or without preceding chemotherapy, were considered in patients with sustained donor engraftment and recurrent hematological malignancy in cells of host origin[83] whereas different strategies, such as second HCT or disease-targeted therapies, were considered for other patients.

Summary and conclusion

Engraftment kinetics after nonmyeloablative or reduced-intensity conditioning have depended on the intensity of pretransplant chemotherapy, the intensity of the conditioning regimens, the graft composition, and the postgrafting immunosuppression. Monitoring mixed chimerism among peripheral blood subpopulations early after transplantation identifies patients at risk for graft rejection and for grade II–IV acute GVHD. Achievement of full donor T-cell chimerism is associated with decreased risk of relapse. Further, preliminary observations suggest that high levels of NK-cell donor chimerism early after HCT might predict better progression-free survival. Chimerism levels among granulocytes have been poor predictors for HCT outcomes, but further studies are needed to evaluate the efficacy of chimerism monitoring among cell subtypes enriched for the phenotype of the malignant cells. In addition, the impact of donor chimerism levels among other cell sub-types (such as dendritic cells,[87,88] Langerhans cells,[89] plasmacytoid dendritic cells,[90] mesenchymal cells,[91] or regulatory T-cells[92]) on HCT outcomes after nonmyemoablative conditioning deserves further investigation. Finally, in patients with hematological relapse after HCT, assessing the origin of both malignant cells and T-cells might assist in determining the best therapeutic options.

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References

1 Bader P, Niethammer D, Willasch A, Kreyenberg H, Klingebiel T. How and when should we monitor chimerism after allogeneic stem cell transplantation? (Review). *Bone Marrow Transplant* 2005; **35**: 107–119.
2 Antin JH, Childs R, Filipovich AH, Giralt S, Mackinnon S, Spitzer T *et al.* Establishment of complete and mixed donor chimerism after allogeneic lymphohematopoietic transplantation: recommendations from a workshop at the 2001 Tandem Meetings (Review). *Biol Blood Marrow Transplant* 2001; **7**: 473–485.
3 Santos GW, Sensenbrenner LL, Burke PJ, Colvin M, Owens Jr AH, Bias WB *et al.* Marrow transplantation in man following cyclophosphamide. *Transplant Proc* 1971; **3**: 400–404.
4 Hill RS, Petersen FB, Storb R, Appelbaum FR, Doney K, Dahlberg S *et al.* Mixed hematologic chimerism after allogeneic marrow transplantation for severe aplastic anemia is associated with a higher risk of graft rejection and a lessened incidence of acute graft-versus-host disease. *Blood* 1986; **67**: 811–816.
5 Huss R, Deeg HJ, Gooley T, Bryant E, Leisenring W, Clift R *et al.* Effect of mixed chimerism on graft-versus-host disease, disease recurrence, and survival after HLA-identical marrow transplantation for aplastic anemia or chronic myelogenous leukemia. *Bone Marrow Transplant* 1996; **18**: 767–776.
6 Branch DR, Gallagher MT, Forman SJ, Winkler KJ, Petz LD, Blume KG. Endogenous stem cell repopulation resulting in mixed hematopoietic chimerism following total body irradiation and

SPOTLIGHT

marrow transplantation for acute leukemia. *Transplantation* 1982; **34**: 226–228.
7 Bertheas MF, Lafage M, Levy P, Blaise D, Stoppa AM, Viens P *et al.* Influence of mixed chimerism on the results of allogeneic bone marrow transplantation for leukemia. *Blood* 1991; **78**: 3103–3106.
8 Petz LD. Documentation of engraftment and characterization of chimerism following bone marrow transplantation. In: Forman SJ, Blume KG, Thomas ED (eds). *Bone Marrow Transplantation*. Blackwell Scientific Publications: Boston, MA, 1994, pp 136–148.
9 Bretagne S, Vidaud M, Kuentz M, Cordonnier C, Henni T, Vinci G *et al.* Mixed blood chimerism in T cell-depleted bone marrow transplant recipients: Evaluation using DNA polymorphisms. *Blood* 1987; **70**: 1692–1695.
10 Roy DC, Tantravahi R, Murray C, Dear K, Gorgone B, Anderson KC *et al.* Natural history of mixed chimerism after bone marrow transplantation with CD6-depleted allogeneic marrow: a stable equilibrium. *Blood* 1990; **75**: 296–304.
11 Schaap N, Schattenberg A, Mensink E, Preijers F, Hillegers M, Knops R *et al.* Long-term follow-up of persisting mixed chimerism after partially T cell-depleted allogeneic stem cell transplantation. *Leukemia* 2002; **16**: 13–21.
12 Mackinnon S, Barnett L, Heller G, O'Reilly RJ. Minimal residual disease is more common in patients who have mixed T-cell chimerism after bone marrow transplantation for chronic myelogenous leukemia. *Blood* 1994; **83**: 3409–3416.
13 Baron F, Sandmaier BM. Current status of hematopoietic stem cell transplantation after nonmyeloablative conditioning. *Curr Opin Hematol* 2005; **12**: 435–443.
14 Baron F, Storb R. Allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning as treatment for hematologic malignancies and inherited blood disorders (Review). *Mol Ther* 2006; **13**: 26–41.
15 Childs RW, Barrett J. Nonmyeloablative allogeneic immunotherapy for solid tumors (Review). *Annu Rev Med* 2004; **55**: 459–475.
16 Giralt S, Estey E, Albitar M, van Besien K, Rondón G, Anderlini P *et al.* Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood* 1997; **89**: 4531–4536.
17 Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, Cividalli G *et al.* Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood* 1998; **91**: 756–763.
18 McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG *et al.* Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood* 2001; **97**: 3390–3400.
19 Baron F, Maris MB, Sandmaier BM, Storer BE, Sorror M, Diaconescu R *et al.* Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol* 2005; **23**: 1993–2003.
20 Mohty M, de Lavallade H, Ladaique P, Faucher C, Vey N, Coso D *et al.* The role of reduced intensity conditioning allogeneic stem cell transplantation in patients with acute myeloid leukemia: a donor vs no donor comparison. *Leukemia* 2005; **19**: 916–920.
21 Shimoni A, Kröger N, Zabelina T, Ayuk F, Hardan I, Yeshurun M *et al.* Hematopoietic stem-cell transplantation from unrelated donors in elderly patients (age >55 years) with hematologic malignancies: older age is no longer a contraindication when using reduced intensity conditioning. *Leukemia* 2005; **19**: 7–12.
22 Burroughs L, Storb R. Low-intensity allogeneic hematopoietic stem cell transplantation for myeloid malignancies: separating graft-versus-leukemia effects from graft-versus-host disease. *Curr Opin Hematol* 2005; **12**: 45–54.
23 Champlin R, Khouri I, Shimoni A, Gajewski J, Kornblau S, Molldrem J *et al.* Harnessing graft-versus-malignancy: non-myeloablative preparative regimens for allogeneic haematopoietic transplantation, an evolving strategy for adoptive immunotherapy. *Br J Haematol* 2000; **111**: 18–29.
24 Storb RF, Champlin R, Riddell SR, Murata M, Bryant S, Warren EH. Non-myeloablative transplants for malignant disease. In: Schechter GP, Broudy VC, Williams ME (eds). *Hematology 2001: American Society of Hematology Education Program Book*. The American Society of Hematology: Washington, DC, 2001, pp 375–391.
25 Appelbaum FR. Dose intensity and the toxicity and efficacy of allogeneic hematopoietic cell transplantation (Keynote Address). *Leukemia* 2005; **19**: 171–175.
26 de Lima M, Anagnostopoulos A, Munsell M, Shahjahan M, Ueno N, Ippoliti C *et al.* Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. *Blood* 2004; **104**: 865–872.
27 Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, Leitman S *et al.* Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. *Blood* 1999; **94**: 3234–3241.
28 Dey BR, McAfee S, Colby C, Sackstein R, Saidman S, Tarbell N *et al.* Impact of prophylactic donor leukocyte infusions on mixed chimerism, graft-versus-host disease, and antitumor response in patients with advanced hematologic malignancies treated with nonmyeloablative conditioning and allogeneic bone marrow transplantation. *Biol Blood Marrow Transplant* 2003; **9**: 320–329.
29 Carvallo C, Geller N, Kurlander R, Srinivasan R, Mena O, Igarashi T *et al.* Prior chemotherapy and allograft CD34+ dose impact donor engraftment following nonmyeloablative allogeneic stem cell transplantation in patients with solid tumors. *Blood* 2004; **103**: 1560–1563.
30 Baron F, Baker JE, Storb R, Gooley TA, Sandmaier BM, Maris MB *et al.* Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. *Blood* 2004; **104**: 2254–2262.
31 Ueno NT, Cheng YC, Rondon G, Tannir NM, Gajewski JL, Couriel DR *et al.* Rapid induction of complete donor chimerism by the use of a reduced-intensity conditioning regimen composed of fludarabine and melphalan in allogeneic stem cell transplantation for metastatic solid tumors. *Blood* 2003; **102**: 3829–3836.
32 Bryant E, Martin PJ. Documentation of engraftment and characterization of chimerism following hematopoietic cell transplantation. In: Blume KG, Forman SJ, Appelbaum FR (eds). *Thomas' Hematopoietic Cell Transplantation*. Blackwell Publishing Ltd.: Oxford, UK, 2004, pp 234–243.
33 Thiede C. Diagnostic chimerism analysis after allogeneic stem cell transplantation: new methods and markers (Review). *Am J PharmacoGenom* 2004; **4**: 177–187.
34 Hochberg EP, Ritz J. Hematpoietic chimerism after allogeneic stem cell transplantation. In: Atkinson K, Champlin R, Ritz J, Fibbe WE, Ljungman P, Brenner MK (eds). *Clinical Bone Marrow and Blood Stem Cell Transplantation*. Cambridge University Press: Cambridge, UK, 2004, pp 466–478.
35 Durnam DM, Anders KR, Fisher L, O'Quigley JO, Bryant EM, Thomas ED. Analysis of the origin of marrow cells in bone marrow transplant recipients using a Y-chromosome-specific *in situ* hybridization assay. *Blood* 1989; **74**: 2220–2226.
36 Mackinnon S, Barnett L, Bourhis JH, Black P, Heller G, O'Reilly RJ. Myeloid and lymphoid chimerism after T-cell-depleted marrow transplantation: evaluation of conditioning regimens using the polymerase chain reaction to amplify human minisatellite regions of genomic DNA. *Blood* 1992; **80**: 3235–3241.
37 Thiede C, Bornhauser M, Ehninger G. Evaluation of STR informativity for chimerism testing – comparative analysis of 27 STR systems in 203 matched related donor recipient pairs. *Leukemia* 2004; **18**: 248–254.
38 Thiede C, Florek M, Bornhäuser M, Ritter M, Mohr B, Brendel C *et al.* Rapid quantification of mixed chimerism using multiplex amplification of short tandem repeat markers and fluorescence detection. *Bone Marrow Transplant* 1999; **23**: 1055–1060.
39 Scharf SJ, Smith AG, Hansen JA, McFarland C, Erlich HA. Quantitative determination of bone marrow transplant engraftment using fluorescent polymerase chain reaction primers for human identity markers. *Blood* 1995; **85**: 1954–1963.
40 Schichman SA, Suess P, Vertino AM, Gray PS. Comparison of short tandem repeat and variable number tandem repeat genetic markers for quantitative determination of allogeneic bone marrow

SPOTLIGHT

transplant engraftment. *Bone Marrow Transplant* 2002; **29**: 243–248.
41 Hancock JP, Goulden NJ, Oakhill A, Steward CG. Quantitative analysis of chimerism after allogeneic bone marrow transplantation using immunomagnetic selection and fluorescent microsatellite PCR (Review). *Leukemia* 2003; **17**: 247–251.
42 Kroger N, Zagrivnaja M, Schwartz S, Badbaran A, Zabelina T, Lioznov M *et al.* Kinetics of plasma-cell chimerism after allogeneic stem cell transplantation by highly sensitive real-time PCR based on sequence polymorphism and its value to quantify minimal residual disease in patients with multiple myeloma. *Exp Hematol* 2006; **34**: 688–694.
43 McSweeney PA, Storb R. Mixed chimerism: preclinical studies and clinical applications (Review). *Biol Blood Marrow Transplant* 1999; **5**: 192–203.
44 Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C *et al.* Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. *Blood* 2002; **99**: 4618–4625.
45 Fredriksson M, Barbany G, Liljedahl U, Hermanson M, Kataja M, Syvanen AC. Assessing hematopoietic chimerism after allogeneic stem cell transplantation by multiplexed SNP genotyping using microarrays and quantitative analysis of SNP alleles. *Leukemia* 2004; **18**: 255–266.
46 Masmas TN, Madsen HO, Petersen SL, Ryder LP, Svejgaard A, Alizadeh M *et al.* Evaluation and automation of hematopoietic chimerism analysis based on real-time quantitative polymerase chain reaction. *Biol Blood Marrow Transplant* 2005; **11**: 558–566.
47 Hogan WJ, Little M-T, Zellmer E, Friedetzky A, Diaconescu R, Gisburne S *et al.* Postgrafting immunosuppression with sirolimus and cyclosporine facilitates stable mixed hematopoietic chimerism in dogs given sublethal total body irradiation before marrow transplantation from DLA-identical littermates. *Biol Blood Marrow Transplant* 2003; **9**: 489–495.
48 Kahl C, Mielcarek M, Iwata M, Harkey MA, Storer B, Torok-Storb B. Radiation dose determines the degree of myeloid engraftment following nonmyeloablative stem cell transplantation. *Biol Blood Marrow Transplant* 2004; **10**: 826–833.
49 Panse JP, Heimfeld S, Guthrie KA, Maris MB, Maloney DG, Baril BB *et al.* Allogeneic peripheral blood stem cell graft composition affects early T-cell chimaerism and later clinical outcomes after nonmyeloablative conditioning. *Br J Haematol* 2005; **128**: 659–667.
50 Tykodi SS, Warren EH, Thompson JA, Riddell SR, Childs RW, Otterud BE *et al.* Allogeneic hematopoietic cell transplantation for metastatic renal cell carcinoma after nonmyeloablative conditioning: toxicity, clinical response, and immunological response to minor histocompatibility antigens. *Clin Cancer Res* 2004; **10**: 7799–7811.
51 Valcarcel D, Martino R, Caballero D, Mateos MV, Perez-Simon JA, Canals C *et al.* Chimerism analysis following allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning. *Bone Marrow Transplant* 2003; **31**: 387–392.
52 Bornhauser M, Thiede C, Platzbecker U, Jenke A, Helwig A, Plettig R *et al.* Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. *Clin Cancer Res* 2001; **7**: 2254–2262.
53 Maris MB, Niederwieser D, Sandmaier BM, Storer B, Stuart M, Maloney D *et al.* HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood* 2003; **102**: 2021–2030.
54 Baron F, Maris MB, Storer BE, Sandmaier BM, Stuart MJ, McSweeney PA *et al.* HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with chronic myeloid leukemia. *Biol Blood Marrow Transplant* 2005; **11**: 272–279.
55 Kerbauy FR, Storb R, Hegenbart U, Gooley T, Shizuru J, Al-Ali HK *et al.* Hematopoietic cell transplantation from HLA-identical sibling donors after low-dose radiation-based conditioning for treatment of CML. *Leukemia* 2005; **19**: 990–997.
56 Girgis M, Hallemeier C, Blum W, Brown R, Lin H-S, Khoury H *et al.* Chimerism and clinical outcomes of 110 unrelated donor bone marrow transplants who underwent conditioning with low-dose, single-exposure total body irradiation and cyclophosphamide. *Blood* 2005; **105**: 3035–3041.
57 Wilson H-MP, Lesnikov V, Plymate SR, Ward J, Deeg HJ. High IGFBP-3 levels in marrow plasma in early-stage MDS: effects on apoptosis and hemopoiesis. *Leukemia* 2005; **19**: 580–585.
58 Faucher C, Mohty M, Vey N, Gaugler B, Bilger K, Moziconnacci MJ *et al.* Bone marrow as stem cell source for allogeneic HLA-identical sibling transplantation following reduced-intensity preparative regimen. *Exp Hematol* 2003; **31**: 873–880.
59 Cao TM, Shizuru JA, Wong RM, Sheehan K, Laport GG, Stockerl-Goldstein KE *et al.* Engraftment and survival following reduced-intensity allogeneic peripheral blood hematopoietic cell transplantation is affected by $CD8^+$ T-cell dose. *Blood* 2005; **105**: 2300–2306.
60 Baron F, Maris MB, Storer BE, Sandmaier BM, Panse JP, Chauncey TR *et al.* High doses of transplanted $CD34^+$ cells are associated with rapid T-cell engraftment and lessened risk of graft rejection, but not more graft-versus-host disease after nonmyeloablative conditioning and unrelated hematopoietic cell transplantation. *Leukemia* 2005; **19**: 822–828.
61 Maris MB, Sandmaier BM, Storer BE, Maloney DG, Shizuru JA, Agura E *et al.* Unrelated donor granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell transplantation after nonmyeloablative conditioning: the effect of postgrafting mycophenolate mofetil dosing. *Biol Blood Marrow Transplant* 2006; **12**: 454–465.
62 Giaccone L, McCune JS, Maris MB, Gooley TA, Sandmaier BM, Slattery JT *et al.* Pharmacodynamics of mycophenolate mofetil after nonmyeloablative conditioning and unrelated donor hematopoietic cell transplantation. *Blood* 2005; **106**: 4381–4388.
63 Matthes-Martin S, Lion T, Haas OA, Frommlet F, Daxberger H, Konig M *et al.* Lineage-specific chimaerism after stem cell transplantation in children following reduced intensity conditioning: potential predictive value of NK cell chimaerism for late graft rejection. *Leukemia* 2003; **17**: 1934–1942.
64 Devetten MP, Vose JM. Graft-versus-host disease: how to translate new insights into new therapeutic strategies (Review). *Biol Blood Marrow Transplant* 2004; **10**: 815–825.
65 Mohty M, Blaise D, Faucher C, Vey N, Bouabdallah R, Stoppa A-M *et al.* Inflammatory cytokines and acute graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation. *Blood* 2005; **106**: 4407–4411.
66 Pelot MR, Pearson DA, Swenson K, Zhao G, Sachs J, Yang Y-G *et al.* Lymphohematopoietic graft-vs-host reactions can be induced without graft-vs-host disease in murine mixed chimeras established with a cyclophosphamide-based nonmyeloablative conditioning regimen. *Biol Blood Marrow Transplant* 1999; **5**: 133–143.
67 Baron F, Little M-T, Storb R. Kinetics of engraftment following allogeneic hematopoietic cell transplantation with reduced-intensity or nonmyeloablative conditioning. *Blood Rev* 2005; **19**: 153–164.
68 Mielcarek M, Martin PJ, Leisenring W, Flowers MED, Maloney DG, Sandmaier BM *et al.* Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood* 2003; **102**: 756–762.
69 Couriel DR, Saliba RM, Giralt S, Khouri I, Andersson B, de Lima M *et al.* Acute and chronic graft-versus-host disease after ablative and nonmyeloablative conditioning for allogeneic hematopoietic transplantation. *Biol Blood Marrow Transplant* 2004; **10**: 178–185.
70 Sorror M, Maris M, Diaconescu R, Storb R. Lessened severe graft-versus-host after 'minitransplantations' (Letter to the Editor). *Blood* 2005; **105**: 2614.
71 Aoudjhane M, Labopin M, Gorin NC, Shimoni A, Ruutu T, Kolb H-J *et al.* Comparative outcome of reduced intensity and myeloablative conditioning regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukaemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT). *Leukemia* 2005; **19**: 2304–2312.
72 Scott BL, Sandmaier BM, Storer B, Maris MB, Sorror ML, Maloney DG *et al.* Myeloablative vs nonmyeloablative allogeneic transplantation for patients with myelodysplastic syndrome or acute myelogenous leukemia with multilineage dysplasia: a retrospective analysis. *Leukemia* 2006; **20**: 128–135.

73 Perez-Simon JA, Diez-Campelo M, Martino R, Sureda A, Caballero D, Canizo C *et al.* Impact of CD34+ cell dose on the outcome of patients undergoing reduced-intensity-conditioning allogeneic peripheral blood stem cell transplantation. *Blood* 2003; **102**: 1108–1113.
74 Mattsson J, Uzunel M, Brune M, Hentschke P, Barkholt L, Stierner U *et al.* Mixed chimaerism is common at the time of acute graft-versus-host disease and disease response in patients receiving non-myeloablative conditioning and allogeneic stem cell transplantation. *Br J Haematol* 2001; **115**: 935–944.
75 Perez-Simon JA, Caballero D, Diez-Campelo M, Lopez-Perez R, Mateos G, Canizo C *et al.* Chimerism and minimal residual disease monitoring after reduced intensity conditioning (RIC) allogeneic transplantation (Review). *Leukemia* 2002; **16**: 1423–1431.
76 Uzunel M, Mattsson J, Brune M, Johansson JE, Aschan J, Ringden O. Kinetics of minimal residual disease and chimerism in patients with chronic myeloid leukemia after nonmyeloablative conditioning and allogeneic stem cell transplantation. *Blood* 2003; **101**: 469–472.
77 Keil F, Prinz E, Moser K, Mannhalter C, Kalhs P, Worel N *et al.* Rapid establishment of long-term culture-initiating cells of donor origin after nonmyeloablative allogeneic hematopoietic stem-cell transplantation, and significant prognostic impact of donor T-cell chimerism on stable engraftment and progression-free survival. *Transplantation* 2003; **76**: 230–236.
78 Passweg JR, Tichelli A, Meyer-Monard S, Heim D, Stern M, Kuhne T *et al.* Purified donor NK-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation. *Leukemia* 2004; **18**: 1835–1838.
79 Marks DI, Parker A, Robinson SP. Donor lymphocyte infusions after reduced intensity conditioning allogeneic transplantation: what we need to know. *Blood* 2004; **104**: 295–296.
80 Lange T, Deininger M, Brand R, Hegenbart U, Al-Ali H, Krahl R *et al. BCR-ABL* transcripts are early predictors for hematological relapse in chronic myeloid leukemia after hematopoietic cell transplantation with reduced intensity conditioning. *Leukemia* 2004; **18**: 1468–1475.
81 van Leeuwen JE, van Tol MJ, Joosten AM, Wijnen JT, Verweij PJ, Khan PM *et al.* Persistence of host-type hematopoiesis after allogeneic bone marrow transplantation for leukemia is significantly related to the recipient's age and/or the conditioning regimen, but it is not associated with an increased risk of relapse. *Blood* 1994; **83**: 3059–3067.
82 Wu CJ, Krishnamurti L, Kutok JL, Biernacki M, Rogers S, Zhang W *et al.* Evidence for ineffective erythropoiesis in severe sickle cell disease. *Blood* 2005; **106**: 3639–3645.
83 Bethge WA, Hegenbart U, Stuart MJ, Storer BE, Maris MB, Flowers MED *et al.* Adoptive immunotherapy with donor lymphocyte infusions after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. *Blood* 2004; **103**: 790–795.
84 Peggs KS, Thomson K, Hart H, Geary J, Morris EC, Yong K *et al.* Dose-escalated donor lymphocyte infusions following reduced intensity transplantation: toxicity, chimerism and disease responses. *Blood* 2004; **103**: 1548–1556.
85 Sala-Torra O, Hanna C, Loken MR, Flowers MED, Maris M, Ladne PA *et al.* Evidence of donor-derived hematologic malignancies after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006; **12**: 511–517.
86 Baron F, Dresse MF, Beguin Y. Transmission of chronic myeloid leukemia through peripheral-blood stem-cell transplantation. *N Engl J Med* 2003; **349**: 913–914.
87 Auffermann-Gretzinger S, Lossos IS, Vayntrub TA, Leong W, Grumet FC, Blume KG *et al.* Rapid establishment of dendritic cell chimerism in allogeneic hematopoietic cell transplant recipients. *Blood* 2002; **99**: 1442–1448.
88 Boeck S, Hamann M, Pihusch V, Heller T, Diem H, Rolf B *et al.* Kinetics of dendritic cell chimerism and T cell chimerism in allogeneic hematopoietic stem cell recipients. *Bone Marrow Transplant* 2006; **37**: 57–64.
89 Collin MP, Hart DN, Jackson GH, Cook G, Cavet J, Mackinnon S *et al.* The fate of human Langerhans cells in hematopoietic stem cell transplantation. *J Exp Med* 2006; **203**: 27–33.
90 Mohty M, Blaise D, Faucher C, Bardou VJ, Gastaut JA, Viens P *et al.* Impact of plasmacytoid dendritic cells on outcome after reduced-intensity conditioning allogeneic stem cell transplantation. *Leukemia* 2005; **19**: 1–6.
91 Poloni A, Leoni P, Buscemi L, Balducci F, Pasquini R, Masia MC *et al.* Engraftment capacity of mesenchymal cells following hematopoietic stem cell transplantation in patients receiving reduced-intensity conditioning regimen. *Leukemia* 2006; **20**: 329–335.
92 Rezvani K, Mielke S, Ahmadzadeh M, Kilical Y, Savani BN, Zeilah J *et al.* High donor *Foxp3*-positive regulatory T-cell (Treg) content is associated with a low risk of GVHD following HLA-matched allogeneic stem cell transplantation (SCT). *Blood; prepublished online Apr* 2006; doi: 10.1182/blood-2206-02-003996.
93 Sykes M, Preffer F, McAfee S, Saidman SL, Weymouth D, Andrews DM *et al.* Mixed lymphohaemopoietic chimerism and graft-versus-lymphoma effects after non-myeloablative therapy and HLA-mismatched bone-marrow transplantation. *Lancet* 1999; **353**: 1755–1759.
94 Dahmen UM, Boettcher M, Krawczyk M, Broelsch CE. Flow cytometric 'rare event analysis': a standardized approach to the analysis of donor cell chimerism. *J Immunol Methods* 2002; **262**: 53–69.