Biology of Blood and Marrow Transplantation 7:473-483 (2001)

REVIEW

Establishment of Complete and Mixed Donor Chimerism After Allogeneic Lymphohematopoietic Transplantation: Recommendations From a Workshop at the 2001 Tandem Meetings

Joseph H. Antin,[1] *Richard Childs*,[2] *Alexandra H. Filipovich*,[3] *Sergio Giralt*,[4] *Stephen Mackinnon*,[5] *Thomas Spitzer*,[6] *Daniel Weisdorf*[7]*

[1]Dana-Farber Cancer Institute, Boston, Massachusetts; [2]National Heart, Lung, and Blood Institute, Bethesda, Maryland; [3]Children's Hospital Medical Center, Cincinnati, Ohio; [4]M. D. Anderson Cancer Center, Houston, Texas; [5]University College London, London, United Kingdom; [6]Massachusetts General Hospital, Boston, Massachusetts; [7]University of Minnesota Medical School, Minneapolis, Minnesota

Correspondence and reprint requests: Joseph H. Antin, MD, Dana-Farber Cancer Institute, 44 Binney St, Boston, MA 02115 (e-mail: jantin@partners.org).

Received June 22, 2001; accepted August 9, 2001

ABSTRACT

Approaches to the measurement of lymphohematopoietic chimerism have evolved from laboratory research to important clinical tools. However, there has been no logical, consistent, and uniform set of recommendations for the measurement of chimerism in clinical transplantation. The National Marrow Donor Program and the International Bone Marrow Transplant Registry (IBMTR) sponsored a workshop to discuss the use of chimerism analysis after allogeneic transplantation. The workshop was organized in an effort to make reasonable recommendations regarding laboratory techniques, the types of specimens to be studied, and the frequency of analysis. The panel recommended the following guidelines:

1. **Chimerism analysis should use sensitive, informative techniques. At present, short tandem repeats (STR) or variable number tandem repeats (VNTR) analysis is the approach most likely to give reproducible informative data.**
2. **Peripheral blood cells are generally more useful than bone marrow cells for chimerism analysis.**
3. **Lineage-specific chimerism should be considered the assay of choice in the setting of nonmyeloablative and reduced-intensity conditioning.**
4. **The use of T-cell depletion, nonmyeloablative or reduced-intensity conditioning, or novel graft-versus-host disease (GVHD) prophylactic regimens warrants chimerism analysis at 1, 3, 6, and 12 months, because interventions such as donor lymphocyte infusions may depend on chimerism status.**
5. **In nonmyeloablative transplantation, the early patterns of chimerism may predict either GVHD or graft loss. Therefore, more frequent (every 2-4 weeks) peripheral blood analysis may be warranted.**
6. **For nonmalignant disorders, chimerism generally should be measured 1, 2, and 3 months after transplantation. Interventions to enhance donor engraftment must be considered on a disease-specific basis in relation to concurrent GVHD and, ultimately, clinical rationale.**

KEY WORDS

Lymphohematopoietic chimerism • Allogeneic transplantation • Restriction fragment length polymorphism

INTRODUCTION

In response to a request from the National Marrow Donor Program and the International Bone Marrow Transplant Registry (IBMTR), a workshop was convened at the annual meeting of the American Society of Blood and Marrow Transplantation (ABMTR) and the IBMTR/ABMTR (2001 Tandem BMT Meetings) to discuss chimerism analysis after allogeneic transplantation. This request was stimulated by the desire to establish a rational and reasonably uniform approach to the routine measurement of lympho-

*Authors are listed in alphabetical order.

Table 1. *Definitions*

Type of Chimerism	Working Definition	Comment
Full chimerism	100% Donor cells detected	Full chimerism implies complete lymphohematopoietic replacement, although most assays are semiquantitative and small numbers of host cells may be undetected.
Mixed chimerism	Host cells are detected in a given cellular compartment, eg, lymphocytes	The literature often lists 5% or 10% donor cells as a criterion for mixed chimerism. We have avoided using a number here, because the sensitivity of measurement techniques varies, and the implication of chimerism depends on the disease and the compartment. In general, any reliable detection of host lymphohematopoietic cells can be considered mixed chimerism.
Split chimerism	One or more whole lineage is host and one or more whole lineage is donor	The details of the split chimerism should be clear, eg, myeloid cells are 100% host and T cells are 100% donor.
Microchimerism	<1% Host cells detected	Microchimerism has primarily been described using highly sensitive techniques in organ transplantation and to evaluate systemic sclerosis. We do not recommend its use after allogeneic stem cell transplantation.

hematopoietic chimerism that could be standardized across treatment centers. Such a system would allow the retrospective analysis of chimerism data by each registry. An expert panel was convened and the workshop was organized in an effort to make reasonable recommendations regarding laboratory techniques, the types of specimens to be studied, and the frequency of analysis. Because of the lack of published (and unpublished) data from systematic evaluations of this area, this set of guidelines is not an evidence-based review. Rather, it represents a consensus view of the authors with input from persons who participated in the workshop.

Definitions

The term chimerism refers to the presence of lymphohematopoietic cells of nonhost origin (Table 1). These cells could be derived incidentally from a fetal-maternal transfusion or a blood transfusion or purposefully after hematopoietic stem cell transplantation (HSCT). Full or complete chimerism generally refers to complete replacement of host by donor lymphohematopoiesis. Mixed chimerism indicates the presence of both donor and recipient cells within a given cellular compartment, eg, lymphocytes. The term split chimerism may be used when one or more compartment is derived wholly from the donor. It is recommended that in describing cases of mixed chimerism the author specify whether the chimerism was detected in whole blood or whether it is lineage specific. In the case of split chimerism, the origin of the lineages studied should be specified. Such terms as monolineage, bilineage, etc, should be avoided in favor of specification of the cell lines involved. The term microchimerism has been used to indicate the presence of donor cells that are detectable only with very sensitive techniques. This entity has been described after organ transplantation [1] and in women with systemic sclerosis in whom small numbers of fetus-derived cells can be detected [2,3].

Since the advent of HSCT more than 30 years ago, it has been recognized that determining the degree to which the donor's lymphohematopoietic system has either superceded or come into equilibrium with the host can be critical to establishing the success of the procedure. Moreover, this determination provides a rational method of assessing the ability of different conditioning regimens, graft-versus-host disease (GVHD), prophylactic regimens, and cellular therapy to promote engraftment and graft-versus-leukemia (GVL) activity. We have learned that these issues are particularly important in patients who receive T-cell–depleted or nonmyeloablative transplants, in which GVL effects may depend on achieving a substantial degree of donor T-cell chimerism. On the other hand, there may be situations in which mixed chimerism may be preferable to full donor chimerism in settings in which full replacement of host lymphohematopoiesis is not important. There may be immunologic benefits to establishing a mixed chimeric state in such diverse situations as organ transplantation, congenital disorders of metabolism, and immunotherapy of solid tumors.

Techniques for Assessment of Chimerism

Very early HSCT studies recognized the importance of establishing chimerism [4], but early investigators had to rely on techniques such as red blood cell phenotyping, immunoglobulin isotype analysis, and cytogenetics to assess the chimeric state [5-9]. Limitations of these techniques include limited degrees of polymorphism, poor sensitivity, and the requirement for a donor and recipient that are sex mismatched.

One technique that is of value in sex-mismatched transplantation is fluorescent in situ hybridization (FISH) for the X and Y chromosomes. This technique is now available in many routine laboratories and is relatively sensitive and quantitative. Established procedures are commercially available, making standardization of the methods possible and ensuring comparable results [10].

The most generally applicable and useful methods to evaluate chimerism are with DNA techniques using restriction fragment length polymorphisms (RFLP) [11]. These polymorphisms are neutral variations in DNA sequence created by either loss or gain of a restriction enzyme cleavage site or by insertion or deletion of DNA between restriction sites. The former are generally 2-allele

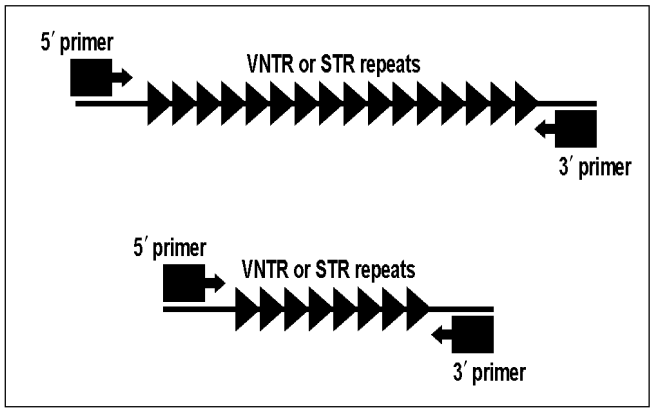

Figure 1. Arrowheads represent DNA repeats, either variable number tandem repeat (VNTR) or short tandem repeat (STR). By using polymerase chain reaction (PCR) primers that flank the repeat sequences, the length of the PCR product will be determined by the number of repeats in each allele.

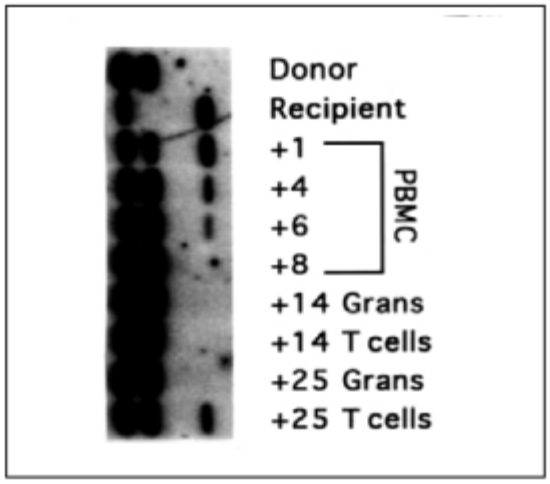

Figure 2. Analysis of minisatellite polymerase chain reaction products following agarose gel electrophoresis, hybridization with ^{32}P-labeled probe, and autoradiography. The figure shows engraftment kinetics following T-cell–depleted allogeneic transplantation. Donor engraftment is established within 8 days posttransplantation, but by day 25 posttransplantation, there is evidence of mixed T-cell chimerism. PBMC indicates peripheral blood mononuclear cells; Grans, granulocytes.

polymorphisms, the latter are often multiallelic if the insertions or deletions result in the formation of human minisatellite (variable number of tandem repeats [VNTR]) or microsatellite (short tandem repeats [STR]) regions of the genome [12]. These loci are inherited in a Mendelian manner and are useful in assessing chimerism after HSCT. Important limitations to conventional RFLP analysis after HSCT are the requirement for at least 10^6 cells to extract sufficient DNA for conventional Southern blot analysis and the lack of sensitivity in detecting minority cell populations. Introduction of the polymerase chain reaction (PCR) as a method for rapid amplification of human minisatellite and microsatellite regions has provided a powerful tool for assessing chimerism [13]. By using PCR primers that flank the minisatellite and microsatellite loci, the whole allele is amplified and therefore the size of the PCR product is determined by the length and number of tandem repeats (Figure 1) [14]. In general, analysis of microsatellites (STR) is simpler than analysis of minisatellites (VNTR). This difference is partly because the repeat regions of many minisatellites are GC rich and partly because minisatellite alleles are larger than microsatellite alleles. Both of these factors make the PCR reaction conditions technically more demanding. The main advantage of a PCR-based method is enhanced sensitivity, which allows for detection of minor populations of donor or recipient cells. In addition, PCR permits analysis from a small number of cells, thus allowing analysis of engraftment kinetics before there is morphological evidence of engraftment. The PCR product can be electrophoresed on an agarose gel, hybridized with ^{32}P-labeled probes, and autoradiographed (Figure 2), or the PCR can be carried out with fluorescently labeled primers and the PCR product visualized using the ABI 310 sequencer (Applied Biosystems, Foster City, CA) (Figure 3). Although the former technique is generally more sensitive, many labs have now switched to using fluorescent primer methodologies because they are faster and avoid the use of radioactivity.

Sensitivity and Quantitation

The sensitivity of chimerism analysis using conventional PCR methods is limited due to the competitive nature of the methodology. In general, the sensitivity is between 0.1% and 5% depending on the allele being tested and whether

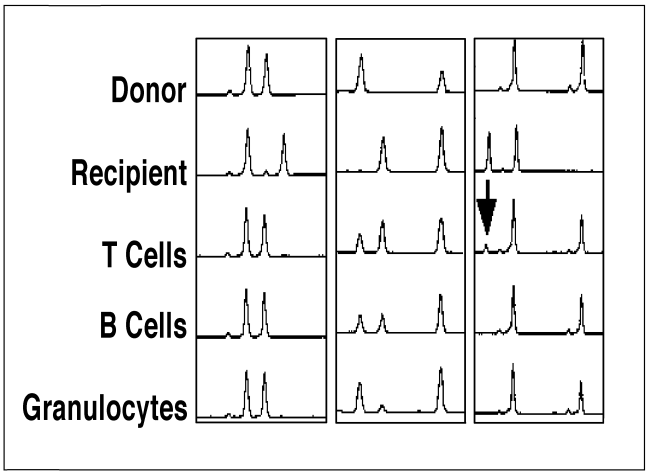

Figure 3. Analysis of fluorescent polymerase chain reaction (PCR) products using the ABI 310 sequencer: lineage-specific chimerism analyses from 3 separate patients following nonmyeloablative transplantation using microsatellite PCR. The arrow indicates persistence of recipient cells. Three separate patterns of chimerism are shown. The left panel shows full donor chimerism following transplantation, the center panel indicates mixed chimerism in all lineages, and the right panel shows mixed chimeras in the T-cell lineage alone, similar to that seen in Figure 2. Stutter peaks can be seen in the donor and recipient alleles in the left and right panels.

Table 2. *Effect of Sample Type and Lineage Selection on Result of Chimerism Analysis*

Patient Sample	Recipient T Cells, %	Chimerism Result
Unseparated marrow	0.6	Donor
Unseparated blood	2	Mixed, lineage unknown
T cells	20	Mixed T cells
Granulocytes	<1	Donor myeloid cells

radioactive or fluorescent detection is being used, as discussed above [15-18]. High levels of sensitivity can be obtained by using Y-specific alleles in a sex-mismatched transplantation (female donor/male recipient), although the prognostic significance of this low-level chimerism is unknown. Quantitation is simple using FISH analysis and can be performed with PCR-based methodologies using minisatellite and microsatellite alleles [15]. In general, most PCR-based methods are semiquantitative, although the degree of quantitation achievable can be increased using multiplex PCR [16,19]. It is possible that the recent introduction of real-time PCR will allow for greater sensitivity and more accurate quantitation in the future. Problems with specificity can arise from "stutter peaks" that result from "slippery" amplification of repeats during PCR amplification [20]. These stutter peaks can be 5% of the size of the corresponding STR or VNTR and may be particularly problematic if the informative recipient allele comigrates with the stutter peak of the donor allele (Figure 3).

Blood Versus Bone Marrow and Unfractionated Versus Lineage-Specific Analysis?

Whether to use blood or bone marrow analysis and whether to use unfractionated or lineage-specific analysis may appear to be 2 separate questions, but, in essence, they address the same issue. There are few instances in which bone marrow chimerism analysis is likely to be more informative than blood chimerism analysis. One is in the detection of minimal residual disease in the bone marrow. As discussed above, chimerism analysis is relatively insensitive—approximately 1% for detecting residual recipient cells. Sensitivity can be increased using lineage-specific analysis; however, this method is usually inferior to disease-specific PCR methodologies and does not usually elucidate whether the residual recipient cells are normal or malignant. In general, most investigators wish to determine first whether donor engraftment has occurred and second whether there is mixed chimerism. If mixed chimerism is present, it may be important to know which lineages are mixed and which are fully donor. With the recent introduction of nonmyeloablative conditioning regimens, it may be important to document both myeloid and lymphoid chimerism. Both may be determined by peripheral blood analysis. Blood may be fractionated into specific lineages by either positive or negative selection techniques. This choice not only allows for analyses of individual lineages but also increases the relative sensitivity of the technique.

For example, T cells may account for only 10% of peripheral blood leukocytes and 3% of bone marrow cells (a not uncommon finding following allogeneic transplantation). If the sensitivity of the method for determining chimerism status is 1% and if 20% of the T cells are of recipient origin and all other lineages are 100% donor, the results shown in Table 2 would be obtained by chimerism analyses of the listed samples. In this example, if the sensitivity of the informative allele were limited to 3% detection of recipient cells (a common observation using fluorescent primers), the analysis of the unfractionated peripheral blood would have spuriously shown complete donor chimerism. Therefore, the use of lineage-specific analyses will increase the sensitivity of the method and allow for accurate assessment of the myeloablative and immunoablative efficacy of different conditioning regimens.

Little is known about mixed B-cell chimerism. It is known to occur after transplantation for some varieties of severe combined immunodeficiency if a myeloablative conditioning regimen is not employed [21]. The presence of mixed B-cell chimerism may be surmised by immunoglobulin allotyping. Analysis using multicolor flow cytometry for such antigens as CD132, (the common cytokine receptor γ chain) or intracellular Wiscott-Aldrich syndrome protein plus CD19 may be useful in future studies.

SUMMARY OF PRESENTATIONS

Assessment of Chimerism Following Myeloablative Transplantation for Hematologic Malignancies

Most of the early studies of chimerism were done in patients receiving myeloablative transplantation for hematologic malignancies. Although mixed chimerism may be detectable using sensitive techniques in some patients, most patients undergoing unmanipulated transplantation will be fully chimeric. Presumably, the T cells in the graft contribute to the establishment of full donor chimerism by a graft-versus-lymphohematopoiesis effect. In contrast, patients receiving marrow that has been T-cell depleted by any of a number of techniques are commonly found to have mixed chimerism [22-25]. Thus, measurement of chimerism is not essential in myeloablative transplantation using conventional conditioning and GVHD prophylaxis. However, if the graft is manipulated, new GVHD regimens are studied, or if the conditioning regimen is altered, the measurement of chimerism may be critical to evaluate the effects of the new regimen.

Assessment of Chimerism Following Nonmyeloablative Stem Cell Transplantation for Lymphoproliferative Disorders

In lymphoproliferative disorders, it is conceivable that a GVL response that eradicates the malignancy does not need to be associated with complete lymphohematopoietic chimerism. Patients who are undertaking allogeneic cell therapy after failed autologous transplantation or as part of a tandem autologous/allogeneic program may be adequately immunocompromised to allow engraftment without additional immunosuppression [26,27]. However, most patients treated early in the course of their disease require additional conditioning. Based on a preclinical murine model in which mixed lymphohematopoietic chimerism is reliably induced following nonmyeloablative preparative therapy and major histocompatibility complex–mismatched bone marrow transplantation [28], the group at Massachusetts General Hospital developed a clinical protocol that incorporates

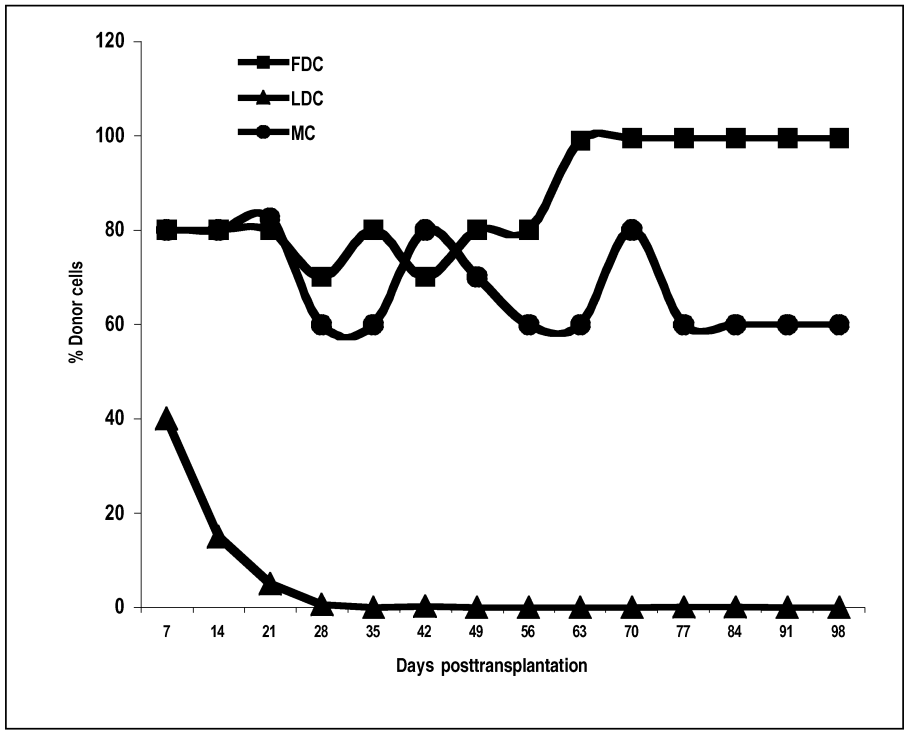

Figure 4. The median percentage of donor cells found in the peripheral blood over time in 41 evaluable recipients of HLA-matched donor hematopoietic stem cell transplantation. Three distinct patterns of chimerism have evolved: Full donor chimerism (FDC) developed either spontaneously or following donor leukocyte infusions (■), sustained mixed chimerism (MC) (●), and loss of donor chimerism (LDC) (▲).

cyclophosphamide, peritransplantation equine antithymocyte globulin or anti-CD2 monoclonal antibody (MEDI-507) therapy, and pretransplantation thymic irradiation (in patients who have not previously received mediastinal irradiation) as preparative therapy for HLA-matched and HLA-mismatched bone marrow transplantation [29,30]. The goal of this strategy was to induce mixed chimerism as an immunological platform for cellular adoptive immunotherapy via donor leukocyte infusions (DLIs). DLIs in this setting are intended to effect a powerful lymphohematopoietic graft-versus-host reaction (with conversion of mixed chimerism to full donor hematopoiesis) without causing significant GVHD.

Sixty-five patients have received an HLA matched (n = 43) or mismatched (n = 22) bone marrow transplant following this nonmyeloablative preparative regimen. Forty-four (68%) of the 65 patients had a diagnosis of advanced non-Hodgkin's lymphoma. Most of these patients had chemotherapy-refractory intermediate grade B–large cell non-Hodgkin's lymphoma, and most patients did not have bone marrow involvement at the time of their transplantation.

Chimerism was measured by PCR analysis of VNTR or STR sequence markers on weekly peripheral blood samples from day 7 through day 100, then every 6 months, on day 28 and day 100, and on yearly bone marrow aspirate samples [31,32]. In the majority of patients, $CD3^+$ T-cell and $CD3^-$ cell fractions were separated by an immunomagnetic cell separation device. For recipients of HLA-mismatched donor transplants, chimerisms of neutrophil, monocyte, and lymphocyte fractions were analyzed by flow cytometry using allele-specific monoclonal anti-HLA antibodies [33].

Results of Chimerism Analyses

All patients initially achieved mixed chimerism. In recipients of HLA-matched donor transplants, 3 patterns of chimerism subsequently evolved (Figure 4). Following a decline in the percentage of donor cells at or shortly after engraftment (coincident with a clinical engraftment syndrome in most patients), the majority of patients achieved persistent mixed chimerism or converted to full donor hematopoiesis (either spontaneously or following "prophylactic" DLI). Approximately 25% of patients ultimately had graft loss.

The results of the chimerism studies correlated well with the clinical sequelae of the transplantations, predicting either risk of GVHD or graft loss (and lack of response to DLI). Analysis of $CD3^+$ T-cell chimerism at the time of intended DLI (day 35 posttransplantation or later) demonstrated that increasing levels of T-cell chimerism were associated with GVHD, whereas T-cell levels of ≤20% were associated with graft loss (despite prophylactic DLI in most cases in which there was declining chimerism).

In recipients of HLA-mismatched donor transplants, mixed chimerism as determined by VNTR/STR and flow cytometric analysis was also initially achieved in all patients. Approximately 85% of patients who received antithymocyte globulin had sustained engraftment. Most of these patients had spontaneous conversion to complete or nearly complete

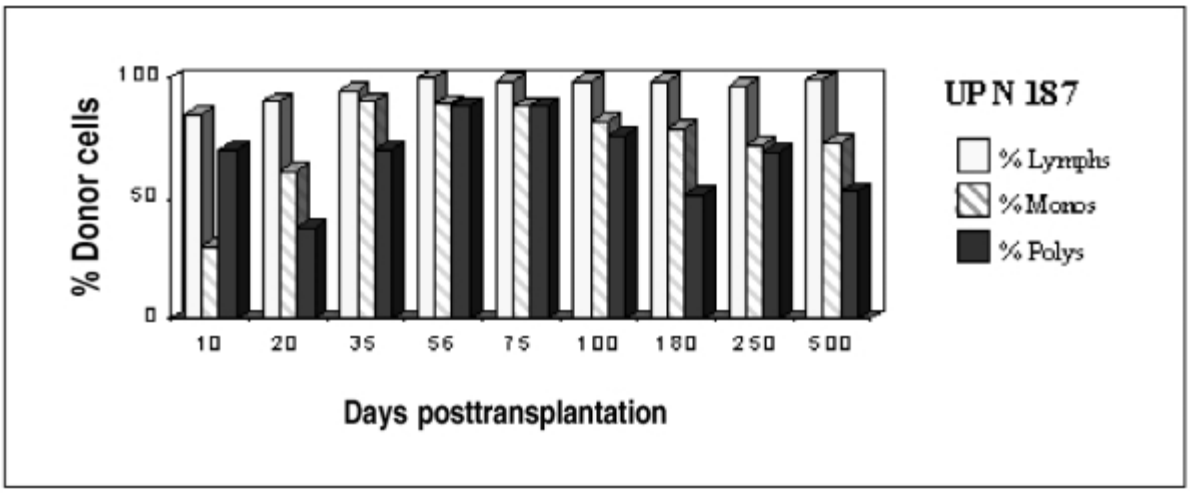

Figure 5. The pattern of mixed chimerism occurring in one patient (UPN 187) who was a recipient of an HLA-mismatched donor transplant showing persistent mixed chimerism in the lymphoid (Lymphs), granulocyte (Polys), and monocyte (Monos) lineages.

donor chimerism (with GVHD). However, persistent mixed chimerism was observed in the granulocyte and monocyte lineages in several cases (Figure 5). Graft loss was seen in an initial cohort of patients receiving an anti-CD2 monoclonal antibody for in vivo T-cell depletion. Following adjustment of the dose and timing of the anti-CD2 monoclonal antibody, sustained donor chimerism was observed in 2 of 3 evaluable patients. VNTR/STR analyses more reliably predicted the clinical courses than flow cytometry; results from the latter method showed spuriously high levels of donor chimerism for several patients. In a comparison of the percentage of donor cells in peripheral blood and bone marrow (at similar time points), no significant difference was observed between the 2 sources of cells (data not shown).

Thus, mixed lymphohematopoietic chimerism can be reliably induced following a nonmyeloablative preparative regimen and HLA-matched– or HLA-mismatched–donor bone marrow transplantation for lymphoproliferative disorders. Prophylactic DLIs have been given to approximately 40% of patients with frequent conversion to full donor hematopoiesis, often with striking antitumor responses. Graft loss, however, has been a problem, particularly in the HLA-matched–donor transplantation setting. In the experience of several other programs employing nonmyeloablative preparative regimens for stem cell transplantation, chimerism has been evaluated infrequently, and, in most situations, full or nearly full donor hematopoiesis was established early [34,35]. The use of DLIs was infrequent and given mostly for persistent or progressive disease.

Chimerism Analysis After Nonmyeloablative and Reduced-Intensity Conditioning for Myeloid Leukemias

A determination of myeloid-specific chimerism is particularly important in patients with malignancies of myeloid origin. Patients with myeloid leukemias in remission who have persistently detectable recipient hematopoiesis, even at extremely low levels, are at considerably higher risk for relapse than those patients who have had a "graft-versus-host hematopoietic effect" resulting in complete donor myeloid chimerism. The assessment of myeloid chimerism, in this setting, appears to offer a "surrogate" approach for determining minimal residual disease, because any detectable host myeloid cell populations would be suspected of harboring malignant cells capable of causing relapse.

Since 1994, the MD Anderson transplantation group has been exploring both nonmyeloablative and reduced-intensity conditioning regimens for allogeneic transplantation in patients with myeloid leukemia. The experience with reduced-intensity conditioning and nonmyeloablative transplantation has been obtained primarily in unsorted bone marrow cells. Because bone marrow samples represent primarily myeloid precursors, chimerism of unsorted bone marrow cells is representative of myeloid lineage chimerism.

Chimerism analysis of unsorted bone marrow cells in the setting of conventional ablative preparative regimen has been reported to predict outcomes such as GVHD, graft failure, and relapse. The results of chimerism analysis of unsorted bone marrow cells for both nonmyeloablative and reduced-intensity conditioning regimens are summarized in Tables 3 and 4.

Table 3. *Chimerism Analysis of Unsorted Bone Marrow Cells in Patients Undergoing Nonmyeloablative (FLAG-Ida) Conditioning Regimens**

	N	Percentage Donor Cells at Day 30 (Range)	Percentage Donor Cells at Day 360 (Range)	Primary/Secondary Autologous Reconstitution
AML/MDS	27	95 (0-100)	100 (0-100)	4/2
CML-CP	7	100 (0-100)	100 (0-100)	1/2
CML-AP	6	45 (0-100)	0	3/6

*FLAG-Ida indicates fludarabine, cytarabine, granulocyte colony–stimulating factor, and idarubicin; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; CP, chronic phase; AP, accelerated phase.

Table 4. *Chimerism Analysis of Unsorted Bone Marrow Cells in Patients Undergoing Reduced Intensity (Fludarabine/Melphalan) Conditioning Regimens*

	N	Mean Percentage of Donor Cells at Day 30	Mean Percentage of Donor Cells at Day 360	Failure/Secondary Autologous Reconstitution, No.
Acute myelogenous leukemia/ myelodysplastic syndrome				
Sibling	**25**	**93.5**	**100**	**1/0**
Unrelated donor	**29**	**93.5**	**100**	**1/0**
Chronic myelogenous leukemia				
Sibling	**16**			**1/1**
Unrelated donor	**18**			**2/1**
Melphalan, 140 mg/m^2				
Yes	**18**	**77.0**	**67**	**2/0**
No	**44**	**99.0**	**100**	**1/0**
Antithymocyte globulin				
Yes	**10**	**75**	**66**	**2/10**
No	**51**	**96**	**100**	**1/51**

Chimerism detected in unsorted bone marrow failed to correlate with any clinical outcomes; however, this result could be due to the small number of patients. However, as seen in Table 5, there was no direct correlation between unsorted bone marrow chimerism and T-cell chimerism. These data demonstrate significant interpatient differences and the inability of unsorted samples to predict for T-cell chimerism. Therefore, lineage-specific chimerism is essential to better understanding of the biologic events that occur after transplantation. In particular, lineage-specific chimerism would allow exploration of the following hypotheses:

1. Lower levels of $CD3^+CD8^+$ donor-cell engraftment will be more strongly associated with rejection than low levels of donor $CD3^+CD4^+$ cells.
2. Low levels of $CD34^+CD38^-$ donor cells will predict graft failure or lack of long-term donor cell engraftment.
3. Mixed chimerism in myeloid lineages will predict relapse in myeloid but not lymphoid malignancies.
4. Mixed chimerism in T-cell subsets will predict a lower risk of acute GVHD.

Assessment of Chimerism After Transplantation for Nonmalignant Disorders

A wide range of nonmalignant disorders are correctable by hematopoietic stem cell transplantation, including immunodeficiencies, acquired and congenital marrow failure syndromes, and hemoglobinopathies. Correction of lethal and degenerative complications of certain systemic metabolic disorders can also be achieved through stable engraftment of hematopoietic cells with normal enzyme activities. For many of these diseases, full hematopoietic chimerism is not required to provide functional reversal or "cure" of the underlying conditions. Thus, conditioning protocols that are believed to be less myeloablative than those containing total body irradiation are often used with transplantation for treatment of such diseases. These regimens are often adequate when treating disorders with defects in lymphocyte function. In contrast, graft rejection is a significant problem in transplantation for treatment of nonmalignant disorders in which immune and marrow functions are robust and no prior chemotherapy has been administered (eg, metabolic disorders), for which even fully ablative conditioning may be inadequate to ensure durable donor cell engraftment.

Long-term mixed chimerism is a frequent consequence of transplantation for treatment of immunodeficiencies that express pathologic lesions in the T-cell lineage, such as severe combined immunodeficiencies (SCIDs), Wiskott-Aldrich syndrome, and hemophagocytic lymphohistiocytosis. Even with conventional "ablative" protocols (eg, busulfan, cyclophosphamide, and etoposide), mixed chimerism in the peripheral blood is seen in approximately 50% of cases. After unrelated or alternative donor transplantation without T-cell depletion, full donor chimerism may be somewhat more common than it is after histocompatible sibling transplantation, perhaps because of a stronger graft-versus-hematopoiesis effect. In these disorders: (1) mixed chimerism prior to day 100 does not predict rejection, (2) chimerism can fluctuate over time without specific interventions, and (3) mixed chimerism is usually compatible with full correction of the T-cell dysfunction, because the circulating T cells are completely, or predominantly, of donor type. Reversal of

Table 5. *Results of Chimerism Analysis of Sorted and Unsorted Samples*

Patient No.	Cell Source	Percentage Donor Cells (Unsorted)	Percentage Donor Cells (T Cell)
13	**Bone marrow**	**6**	**7**
12	**Bone marrow**	**11**	**12**
3	**Bone marrow**	**25**	**73**
7	**Bone marrow**	**30**	**63**
11	**Bone marrow**	**49**	**67**
1	**Bone marrow**	**62**	**72**
9	**Bone marrow**	**64**	**35**
10	**Bone marrow**	**75**	**0**
15	**Bone marrow**	**81**	**75**
8	**Bone marrow**	**85**	**89**
8	**Bone marrow**	**89**	**88**
14	**Bone marrow**	**96**	**95**
4	**Bone marrow**	**99**	**99**

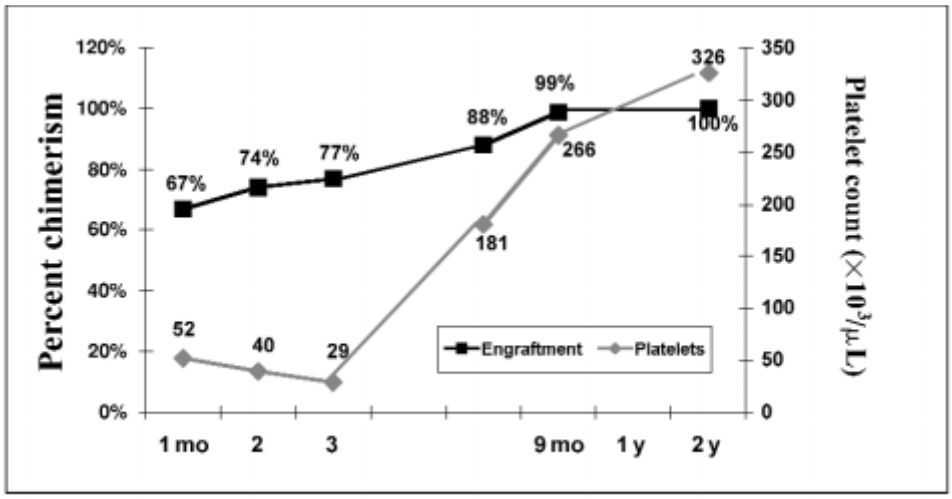

Figure 6. An 11-month-old patient with Wiskott-Aldrich syndrome received a 5/6 unrelated cord blood transplant. Initial mixed chimerism spontaneously converted to full donor chimerism over a year's time, with concurrent recovery of normal platelet count.

proportional donor-type engraftment from 5% to 100% has been observed. Generally speaking, engraftment of >10% donor cells may be adequate for functional correction of the underlying disease. An example of gradual increase in donor chimerism (without specific intervention) is illustrated in Figure 6, which represents the course of an 11-month-old boy who received an unrelated-donor cord blood transplant following conventional myeloablative chemotherapy for Wiskott-Aldrich syndrome.

For congenital defects of nonlymphoid hematologic lineages (eg, chronic granulomatous disease, leukocyte adhesion deficiency, and sickle cell disease), sustained low levels of donor-type hematopoiesis may suffice to reverse the clinical consequences of the inborn errors, assuming that some donor-type neutrophils or erythrocytes are continuously produced. Donor engraftment in the range of 3% to 10% may be clinically useful.

In systemic metabolic disorders, such as Hurler's syndrome or X-linked adrenoleukodystrophy, the consequences of mixed hematopoietic chimerism are less well defined. Early recovery of host type cells after transplantation is often associated with eventual complete graft rejection, whereas late onset of mixed chimerism may have no discernible deleterious effect. There is growing evidence that stabilization of neurologic disease depends on the earliest possible delivery of adequate concentrations of corrective enzyme to the neural tissues by circulating and tissue-specific donor-derived hematopoietic cells. Thus, full hematopoietic chimerism following transplantation from a homozygous normal donor is preferred. Figure 7 demonstrates successful reversal of declining

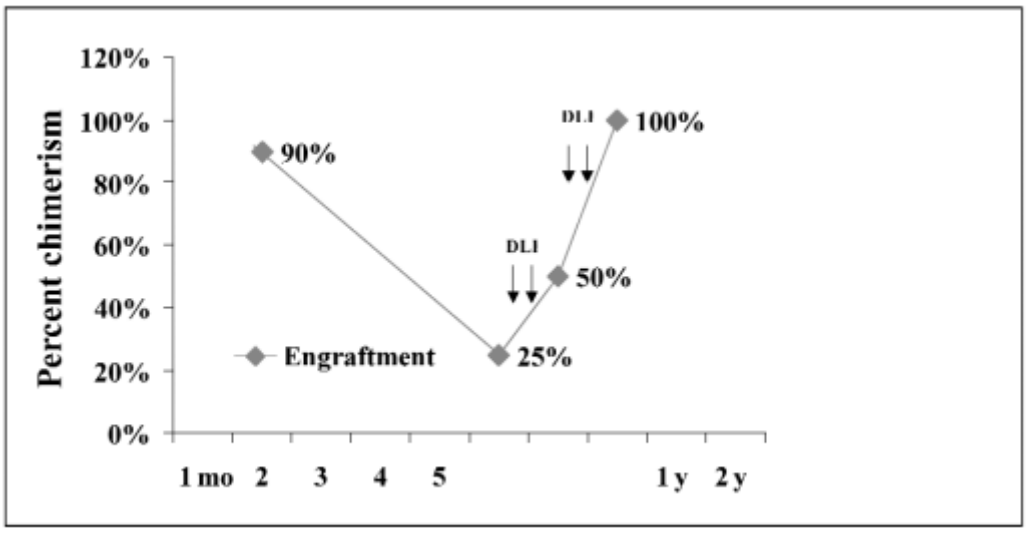

Figure 7. A 2-year-old male patient received a 5/6-matched related donor hematopoietic stem cell transplant (HSCT) for Hurler's syndrome. To reverse declining donor engraftment, donor lymphocyte infusions (DLI) were administered 6.5 and 10 months post-HSCT, resulting in full donor chimerism 10 months after marrow transplantation.

Table 6. *Donor Chimerism Levels Desirable for Functional Correction of Genetic Disorders**

Percentage Donor Cells†	Diseases	Comments
3-10	Sickle cell anemia	RBC graft required
	Chronic granulomatous disease	PMN graft required
	Leukocyte adhesion deficiency	PMN, lymphoid graft required
>10	Severe combined immunodeficiency	Usually T cells‡
	Wiskott-Aldrich syndrome	Platelet graft required
	CD40 ligand deficiency	
	Hemophagocytic lymphohistiocytosis	NK-cell, CTL graft required
100	Autoimmune lymphoproliferative syndrome	
	Fanconi's anemia	

*RBC indicates red blood cells; PMN, polymorphonucleocytes; NK, natural killer; CTL, cytotoxic T lymphocyte.

†Peripheral blood nucleated cells.

‡Many patients require lifetime intravenous immunoglobulin G because of lack of B-cell engraftment.

donor type engraftment through the use of donor lymphocyte infusions from the 5/6-matched related bone marrow donor administered to a child with Hurler's syndrome who had received a myeloablative transplantation regimen.

Systemic disorders of chromosomal instability are due to defects in cell cycle regulation or apoptosis (eg, Fanconi's anemia, Bloom's syndrome, and autoimmune lymphoproliferative disorders). These disorders predispose patients to hematologic malignancies, therefore, full hematopoietic engraftment is desirable.

Transplantation for aplastic anemia using a matched sibling donor is usually undertaken with nonmyeloablative conditioning regimens consisting of cyclophosphamide or cyclophosphamide with antithymocyte globulin. Radiation-based regimens are now primarily used in mismatched or unrelated donor transplantation. There is a high likelihood of encountering mixed chimerism after successful transplantation for treatment of severe aplastic anemia using less intense conditioning regimens. Indeed, occasional patients may have complete recovery of host lymphohematopoiesis [36,37]. At present, it is unknown whether the use of peripheral blood stem cells rather than bone marrow will affect the overall incidence of mixed chimerism. Monitoring chimerism after transplantation for aplastic anemia may be useful in the assessment of graft loss and relapse, but as long as hematopoiesis is sustained, the presence of mixed chimerism may not be clinically relevant.

A number of problems have been seen with persistent mixed chimerism in nonmalignant disorders. These include: (1) compartmental engraftment or split chimerism, eg, in Wiskott-Aldrich syndrome, when donor lymphoid engraftment exists, but defective host thrombopoiesis persists; (2) alloimmunization—donor lymphocyte engraftment with antibody-mediated antihost cytopenias; and (3) persistence of host stem cells that have been exposed to potentially transforming chemotherapies during the transplantation procedure and eventually give rise to hematologic malignancies. All of these complications represent transient or long-term failures of the transplantation procedures.

Table 6 summarizes proportional chimerism deemed adequate for long-term functional correction of nonmalignant genetic diseases after HSCT. Evidence to date suggests that the key factors that can favorably influence durable and more complete donor-type engraftment in nonmalignant genetic disorders include, in approximate descending order of importance: larger graft cell dose, tolerable host/donor histoincompatibility (without extensive T-cell depletion), and intensity of conditioning regimen. Interventions after the HSCT, such as early discontinuation of cyclosporine prophylaxis and induction of GVHD, can sometimes lead to establishment of donor cell predominance. With the current interest in scaling back conditioning protocols for nonmalignant disorders, frequent testing for chimerism is required to determine when immune suppression should be withdraw and/or additional donor lymphocytes should be infused to attempt to stabilize the declining donor graft.

Chimerism Assessment Following Nonmyeloablative Hematopoietic Stem Cell Transplantation for Solid Tumors

For the treatment of solid tumors, the preparative regimen often provides minimal to no direct antimalignancy effect. Therefore, there is a greater need to rapidly establish donor immune engraftment to a level sufficient to confer a graft-versus-tumor (GVT) effect. Chimerism assessment may be used to successfully guide methods to manipulate donor immune reconstitution to enhance a GVT effect (ie, modifying posttransplantation immunosuppression and/or the infusion of donor lymphocytes).

The rate and degree of donor engraftment following HSCT may vary considerably between different cellular lineages and are directly affected by factors such as the patient's pretransplantation immune status as well as the choice and dose of agents used for conditioning and GVHD prophylaxis. The risk of graft rejection following HSCT in patients with a prior history of autologous transplantation is low [26,27]. Rather, such heavily immunosuppressed patients are at risk for early and severe acute GVHD as a consequence of rapid and complete donor immune engraftment. In contrast, patients with little to no history of prior chemotherapy (ie, solid tumor patients, chronic myeloid leukemia patients) undergoing HSCT frequently have more delayed donor engraftment and appear to be at higher risk for graft rejection than patients who have received multiple prior chemotherapeutic regimens.

The critical impact that different conditioning agents have on donor engraftment has become increasingly clear over the past few years. For instance, regimens that use

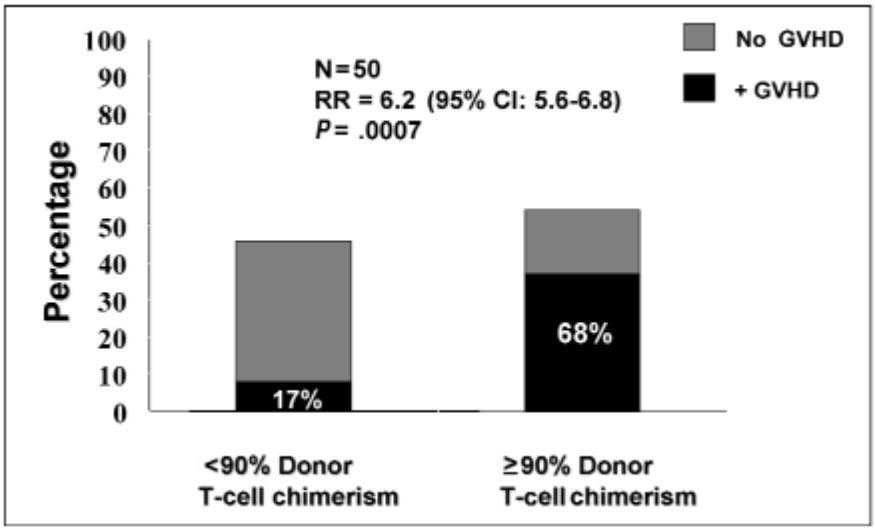

Figure 8. Percentage of patients on day 30 with <90% donor T-cell chimerism or ≥ 90% donor T-cell chimerism. Patients with more rapid (ie, ≥ 90%) donor T-cell engraftment by day 30 were at higher risk for developing acute grade II to IV graft-versus-host disease (GVHD) (68% actuarial risk) than those with more delayed donor T-cell engraftment (17% actuarial risk), shown in black. RR indicates relative risk; CI, confidence interval.

"stem-cell toxic" agents such as melphalan, even at lower doses, are still highly myelosuppressive and are much less likely to permit the high levels of autologous myeloid recovery that are seen with regimens that spare recipient stem cells. In contrast, regimens that use less myelosuppressive (non–stem-cell toxic) but more immunosuppressive agents such as cyclophosphamide and fludarabine are much more likely to result in lower degrees of early donor myeloid engraftment, largely as a consequence of the substantial autologous hematopoietic recovery that ensues. In studies performed at the National Institutes of Health, a cyclophosphamide (120 mg/kg)/fludarabine (125 mg/m^2)-based conditioning regimen followed by infusion of a T-cell replete allograft resulted in considerably higher levels of early donor T-cell engraftment than occurred in donor myeloid lineages [38,39]. Indeed, the observation that full donor T-cell chimerism occurred more promptly than full donor myeloid chimerism in the majority of patients receiving such conditioning provides evidence that cyclophosphamide and fludarabine, given in combination at these doses, are more immunosuppressive than myelosuppressive. Furthermore, when this cyclophosphamide/fludarabine-based nonmyeloablative transplantation protocol was modified to include a T-cell–depleted allograft, the degree of early donor T-cell engraftment declined substantially and was associated with a higher incidence of graft rejection. These observations highlight the critical role that allogeneic lymphocytes play in facilitating donor engraftment following nonmyeloablative HSCT. Furthermore, the observation that low degrees of early donor T-cell engraftment are associated with a higher incidence of graft rejection and a decreased probability of both GVHD and the generation of a GVT effect emphasizes the importance of assessing lineage-specific chimerism for prognostic reasons as well as to permit successful posttransplantation engraftment manipulation.

Whereas most patients with hematological malignancies receive multiple rounds of highly immunosuppressive chemotherapy prior to undergoing allogeneic transplantation, many patients with solid tumors are chemotherapy naive prior to undergoing HSCT. In patients with solid tumors, T-cell chimerism predicted for major alloimmune responses, including the risk of acute GVHD, graft rejection, and the generation of a GVT effect. Patients with high degrees of early donor T-cell chimerism (Figure 8) were significantly more likely to develop acute GVHD than those with lower degrees of early donor T-cell engraftment. Furthermore, acute GVHD typically was not observed until T-cell chimerism had become predominantly donor in origin or during the period when T-cell chimerism was in transition from mixed toward complete donor. Graft rejection occurred in less than 5% of patients and was seen only in patients who had low degrees of early donor T-cell engraftment (typically <50% donor). The onset of a GVT effect was similar to the onset of GVHD, particularly in patients with renal cell carcinoma, in whom the response was often markedly delayed and did not occur until T-cell chimerism was completely donor or was in transition from mixed to complete donor in origin (Figure 9). The assessment of T-cell chimerism, therefore, provides important prognostic information and should be analyzed in this setting.

The optimal methodology to assess chimerism following such procedures is currently uncertain. In solid tumor transplantation there have been no significant differences in myeloid or T-cell chimerism in cellular fractions isolated from the either the peripheral blood or bone marrow. Karyotyping of unsorted bone marrow metaphases in sex-mismatched transplantation has not been useful to assess donor engraftment, because the majority of such metaphase cells are myeloid in origin, providing little insight into the T-cell compartment. In several instances, day 30 bone marrow analysis revealed no evidence for donor-type sex chromosomes in routine metaphase spreads at a time when lineage-specific chimerism (analyzed by VNTR analysis)

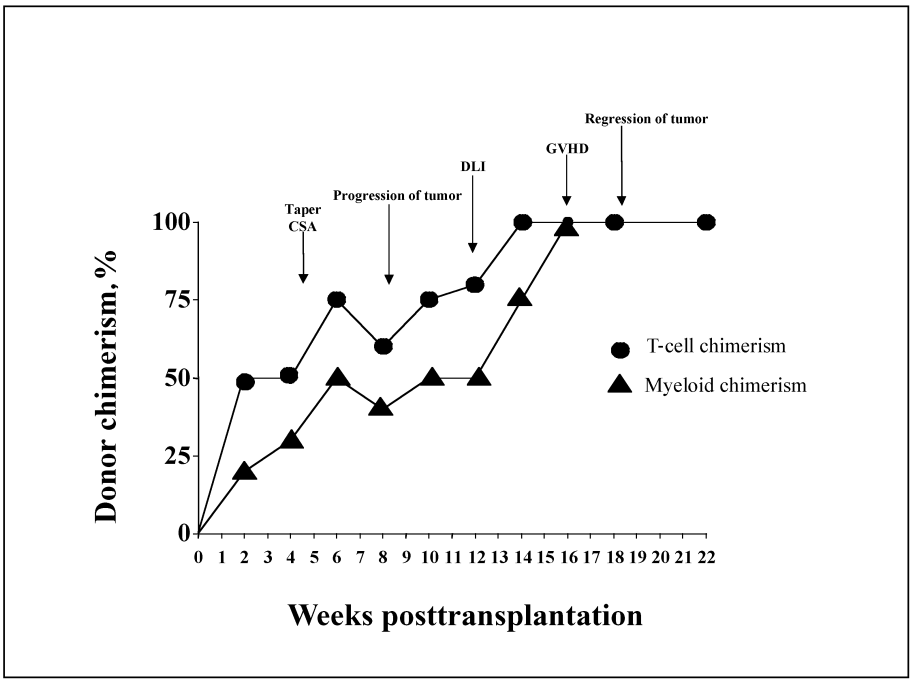

Figure 9. Relationship of donor T-cell and myeloid chimerism to clinical events in a patient with metastatic renal cell carcinoma (RCC) undergoing nonmyeloablative allogeneic peripheral blood stem cell transplantation. High degrees of recipient myeloid and donor T-cell chimerism observed in the first 2 months following transplantation result as a consequence of using nonmyeloablative conditioning, which is more immunosuppressive than myelosuppressive conditioning (fludarabine and cyclophosphamide). Conversion from mixed to complete donor chimerism is achieved following the withdrawal of cyclosporine (CSA) and a donor lymphocyte infusion (DLI) of 1×10^7 $CD3^+$ cells/kg, consistent with a graft-versus-host lymphohematopoietic effect. Acute graft-versus-host disease (GVHD) and RCC disease regression were associated with the transition from mixed to complete donor chimerism compatible with a break in donor tolerance to host antigens.

revealed T cells that were substantially donor in origin. Karyotypic assessment of donor engraftment alone could lead to a false diagnosis of graft rejection, especially with regimens associated with high degrees of autologous myeloid recovery. Hypermetaphase FISH analysis is unlikely to be more helpful in this setting.

RECOMMENDATIONS

1. Chimerism analysis should be undertaken using sensitive (<1%), informative techniques. At present, STR/VNTR analysis is the approach most likely to give reproducible informative data. Cytogenetics, FISH, and hypermetaphase FISH may also be useful if STR/VNTR is unavailable or in sex-mismatched transplantation. However, these techniques are less sensitive. Analyses used for clinical decision-making should be performed in Clinical Laboratory Improvement Amendment (CLIA)-certified laboratories.
2. Peripheral blood stem cells are generally more useful than bone marrow cells for chimerism analysis. Chimerism of unsorted bone marrow cells allows for documentation of origin of myeloid cells; however, it correlates poorly with chimerism of T cells and cells of other lineages and provides little information about degree of chimerism in other cellular subsets. Lineage-specific chimerism should be considered the assay of choice in the setting of nonmyeloablative and reduced-intensity conditioning.
3. Chimerism analysis after myeloablative HSCT using conventional GVHD prophylaxis is probably not essential and can be considered optional. If it is obtained, a schedule of 1, 3, 6, and 12 months is reasonable. Once full chimerism is established, repeat testing is unnecessary unless there is a change in clinical condition. The use of T-cell depletion, nonmyeloablative or reduced-intensity conditioning, or novel GVHD prophylactic regimens warrants chimerism analysis at 1, 3, 6, and 12 months, because interventions such as donor lymphocyte infusions may depend on donor status.
   a. Patients undergoing transplantation for treatment of aplastic anemia should have routine chimerism analysis done on unfractionated blood specimens at 1, 3, 6, and 12 months. Additional testing may be warranted if clinically indicated.
4. In nonmyeloablative transplantation, the early patterns of chimerism may be predictive for either GVHD (increasing donor T-cell chimerism) or graft loss (declining donor T-cell chimerism to ≤20% donor cells). Therefore, if therapeutic intervention is based on these patterns of chimerism, the following recommendations apply to protocols in which achieving mixed chimerism as an immunological platform for DLI is a primary goal:
   a. Frequent (every 2-4 weeks) peripheral blood analysis of chimerism by VNTR or STR analysis until DLI is administered. DLIs are then considered for declining chimerism (but T-cell chimerism greater

than 20%), stable mixed chimerism of >2 weeks duration, or persistent/progressive disease.
   b. For patients who have developed GVHD and are not DLI candidates or who have achieved full donor chimerism, infrequent chimerism analyses are recommended (every 3-6 months).
   c. Multiple chimerism evaluations (initially every 2-4 weeks, then every 3-6 months) are recommended following prophylactic DLI, both to evaluate the effect on chimerism of the DLI and to discern whether additional DLIs are necessary.
5. For nonmalignant disorders other than aplastic anemia, chimerism analysis should generally be done at 1, 2, and 3 months posttransplantation.
   a. If the proportion of donor cells is observed to decline, ongoing monthly follow-up is warranted. Interventions to enhance donor engraftment must be considered on a disease-specific basis and related to concurrent GVHD and, ultimately, clinical rationale.
   b. In some diseases, definition of donor engraftment by hematopoietic lineage is useful in interpreting the transplantation outcomes. Lineage-specific flow-cytometric sorting or other cell-type sorting can be used. For certain immunodeficiencies, flow cytometry can be used to detect donor-derived lymphoid subsets by the presence of expressed cell surface molecules specifically deficient in a given disease, eg, CD132 in X-linked SCID or CD127 in interleukin-7R α–deficient SCID.

ACKNOWLEDGMENT

The authors thank Dr. Mary Horowitz for her thoughtful comments and review of the manuscript

REFERENCES

1. Sahota A, Gao S, Hayes J, Jindal RM. Microchimerism and rejection: a meta-analysis. *Clin Transplant*. 2000;14:345-350.
2. Evans PC, Lambert N, Maloney S, Furst DE, Moore JM, Nelson JL. Long-term fetal microchimerism in peripheral blood mononuclear cell subsets in healthy women and women with scleroderma. *Blood*. 1999;93:2033-2037.
3. Maloney S, Smith A, Furst DE, et al. Microchimerism of maternal origin persists into adult life. *J Clin Invest*. 1999;104:41-47.
4. Mathe G, Amiel JL, Schwartzenberg L, et al. Successful allogenic bone marrow transplantation in man: chimerism, induced specific tolerance and possible anti-leukemic effects. *Blood*. 1965;25:179-195.
5. Walker H, Singer CR, Patterson J, Goldstone AH, Prentice HG. The significance of host haemopoietic cells detected by cytogenetic analysis of bone marrow from recipients of bone marrow transplants. *Br J Haematol*. 1986;62:385-391.
6. Roy DC, Tantravahi R, Murray C, et al. Natural history of mixed chimerism after bone marrow transplantation with CD6-depleted allogeneic marrow: a stable equilibrium. *Blood*. 1990;75:296-304.
7. Lawler SD, Harris H, Millar J, Barrett A, Powles RL. Cytogenetic follow-up studies of recipients of T-cell depleted allogeneic bone marrow. *Br J Haematol*. 1987;65:143-150.
8. Sparkes MC, Crist ML, Sparkes RS, Gale RP, Feig SA. Gene markers in human bone marrow transplantation. *Vox Sang*. 1977;33:202-205.
9. Witherspoon RP, Schanfield MS, Storb R, Thomas ED, Giblett ER. Immunoglobulin production of donor origin after marrow transplantation for acute leukemia or aplastic anemia. *Transplantation*. 1978;26:407-408.
10. Dewald G, Stallard R, Al Saadi A, et al. A multicenter investigation with interphase fluorescence in situ hybridization using X- and Y-chromosome probes. *Am J Med Genet*. 1998;76:318-326.
11. Ginsburg D, Antin JH, Smith BR, Orkin SH, Rappeport JM. Origin of cell populations after bone marrow transplantation. Analysis using DNA sequence polymorphisms. *J Clin Invest*. 1985;75:596-603.
12. Jeffreys A, Wilson V, Thein SL. Hypervariable "minisatellite" regions in human DNA. *Nature*. 1985;314:67-73.
13. Saiki R, Gelfand D, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*. 1988;239:487-491.
14. Jeffreys A, Wilson V, Neumann R, Keyte J. Amplification of human minisatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells. *Nucl Acids Res*. 1988;16:10953-10971.
15. Luhm R, Bellissimo D, Uzgiris A, Drobyski W, Hessner M. Quantitative evaluation of post-bone marrow transplant engraftment status using fluorescent-labeled variable number of tandem repeats. *Mol Diagn*. 2000;5:129-138.
16. Thiede C, Florek M, Bornhauser M, 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.
17. Bader P, Holle W, Klingebiel T, Handgretinger R, Niethammer D, Beck J. Quantitative assessment of mixed hematopoietic chimerism by polymerase chain reaction after allogeneic BMT. *Anticancer Res*. 1996;16:1759-1763.
18. 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.
19. Thiede C, Bornhauser M, Oelschlagel U, et al. Sequential monitoring of chimerism and detection of minimal residual disease after allogeneic blood stem cell transplantation (BSCT) using multiplex PCR amplification of short tandem repeat-markers. *Leukemia*. 2001;15:293-302.
20. Walsh PS, Fildes NJ, Reynolds R. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Res*. 1996;24:2807-2812.
21. Korver K, de Lange GG, van den Bergh RL, et al. Lymphoid chimerism after allogeneic bone marrow transplantation. Y- chromatin staining of peripheral T and B lymphocytes and allotyping of serum immunoglobulins. *Transplantation*. 1987;44:643-650.
22. Bretagne S, Vidaud M, Kuentz M, et al. Mixed blood chimerism in T cell depleted bone marrow transplant recipients: evaluation using DNA polymorphism. *Blood*. 1987;70:1692-1695.
23. 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.
24. Offit K, Burns JP, Cunningham I, et al. Cytogenetic analysis of chimerism and leukemia relapse in chronic myelogenous leukemia patients after T cell-depleted bone marrow transplantation. *Blood*. 1990;75:1346-1355.
25. Bertheas MF, Lafage M, Levy P, et al. Influence of mixed chimerism on the results of allogeneic bone marrow transplantation for leukemia. *Blood*. 1991;78:3103-3106.

26. Porter DL, Luger SM, Duffy KM, et al. Allogeneic cell therapy for patients who relapse after autologous stem cell transplantation. *Biol Blood Marrow Transplant*. 2001;7:230-238.
27. Porter DL, Connors JM, Van Deerlin VM, et al. Graft-versus-tumor induction with donor leukocyte infusions as primary therapy for patients with malignancies. *J Clin Oncol*. 1999;17:1234-1243.
28. Pelot MR, Pearson DA, Swenson K, 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.
29. Sykes M, Preffer F, McAfee S, 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.
30. Spitzer TR, McAfee S, Sackstein R, et al. Intentional induction of mixed chimerism and achievement of antitumor responses after nonmyeloablative conditioning therapy and HLA-matched donor bone marrow transplantation for refractory hematologic malignancies. *Biol Blood Marrow Transplant*. 2000;6:309-320.
31. Schwartz DW, Glock B, Jungl EM, Mayr WR. Strategy to detect chimerism in allogeneic bone marrow transplant recipients by PCR-amplification fragment length polymorphism analysis of microsatellite polymorphisms. *Vox Sang*. 1995;68:139-143.
32. Nakao S, Nakatsumi T, Chuhjo T, et al. Analysis of late graft failure after allogeneic bone marrow transplantation: detection of residual host cells using amplification of variable number of tandem repeats loci. *Bone Marrow Transplant*. 1992;9:107-111.
33. Preffer F. Flow Cytometry. In: Colvin R, Bhan A, McCluskey R, eds. *Diagnostic Immunopathology*. New York, NY: Raven Press; 1995:567-574.
34. Slavin S, Nagler A, Naparstek E, 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.
35. Giralt S, Estey E, Albitar M, 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.
36. Hill RS, Petersen FB, Storb R, 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.
37. Casado LF, Steegmann JL, Pico M, et al. Study of chimerism in long-term survivors after bone marrow transplantation for severe acquired aplastic anemia. *Bone Marrow Transplant*. 1996;18:405-409.
38. Childs R, Chernoff A, Contentin N, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med.* 2000; 343:750-758.
39. Childs R, Clave E, Contentin N, 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