Recurrent disease or donor cell leukemia?

Brain teaser after allogeneic bone marrow transplantation

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Key words: allogeneic bone marrow transplantation, chimerism, donor cell leukemia, relapse, clonal evolution

Abbreviations: allo-BMT, allogeneic bone marrow transplantation; DCL, donor cell leukemia; FISH, fluorescence in situ hybridization; INDEL, short inversion/deletion polymorphism; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism; STR, short tandem repeat; VNTR, variable number of tandem repeats; Y-QPCR, Y-chromosome-specific quantitative polymerase chain reaction

Submitted: 12/29/10

Accepted: 01/05/11

DOI: 10.4161/chim.2.1.14726

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Addendum to: Alpár D, Nagy G, Hohoff C, Kajtár B, Bartyik K, Hermesz J, et al. Sex chromosome changes after sex-mismatched allogeneic bone marrow transplantation can mislead the chimerism analysis. *Pediatr Blood Cancer* 2010; 55:1239–42; PMID: 20979181; DOI: 10.1002/pbc.22617.

Allogeneic bone marrow transplantation (allo-BMT) is the treatment of choice for many patients with poor prognosis or refractory leukemia. Chimerism and residual tumor load after allo-BMT are widely monitored to detect impending graft rejection and the early phase of relapse. In most cases, the malignant cell population during post-transplant relapse contains host-derived cells, but the leukemic clone can rarely be of donor-cell origin. Various genetic tests with different strategies, targets and sensitivities are available for donor-host discrimination. However, changes in the genomic material of the dominant host cell population as a result of clonal evolution and/or clonal selection can hamper the correct identification of the origin of aberrant cells after allo-BMT, thus confounding the assessment of the chimeric state. Consequently, a good knowledge of the techniques applied in clinical practice and careful interpretation of their results are essential. A lack of host-specific markers at the time of clinical relapse is not adequate for verifying the presence of donor cell leukemia, and unequivocal demonstration of donor-specific markers is also required.

Assessment of the chimeric state and minimal residual disease after allogeneic bone marrow transplantation (allo-BMT) provide crucial information on the progress of engraftment, and predict impending graft rejection or relapse.^{1,2} In the majority of cases, the malignant clone appearing during clinical relapse is composed of host cells; however, several patients with donor-cell leukemia (DCL) have also been

reported.^{3–6} Correct identification of the origin of aberrant cells can be difficult in some cases, because of the possible evolution of the original leukemic clone and/or clonal selection of host cells.

We recently published a case report describing a 12-year-old boy with pre-B-cell acute lymphoblastic leukemia with a cryptic *BCR/ABL* rearrangement who underwent sex-mismatched allo-BMT. The patient relapsed three months post-transplant; however, the Y chromosome was lost and the X chromosome was duplicated in the residual leukemic cells, leading to confusing results of chimerism investigations. An important finding of the study was the fact that six out of the eight different techniques applied were unable to unequivocally exclude the possibility of DCL.⁷ This case drew attention to the need for careful interpretation of the results of tests to monitor chimerism.

The first report of a patient with DCL was published by Fialkow, et al. in 1971, and more than 60 cases have been reported to date.^{3,8} Nevertheless, it is difficult to verify the real number of patients with DCL because several were only diagnosed by conventional and/or molecular cytogenetic studies. Furthermore, a subset of the remaining cases was merely confirmed indirectly, i.e., by demonstrating an absence of residual host hematopoiesis. However, the lack of one or more genetic markers identified in the original leukemic clone does not unambiguously demonstrate a donor origin for the dominant aberrant cells at the time of clinical relapse. One of the most impressive evidences for this was provided by Spinelli, et al. who described a male patient with

Table 1. Techniques for estimating the chimeric state

Method	Sensitivity	Advantages	Disadvantages
Karyotyping	10 ⁻¹	Evaluates the whole chromosome assemblage	Low representativity; restricted to dividing cells
FISH	10 ⁻² –10 ⁻³	Cell-based method allowing direct quantification; cell culturing is not a prerequisite	Restricted to sex-mismatched BMT
RFLP	10 ⁻² –10 ⁻³	Sex-independent; smaller sample is needed than for cytogenetic techniques	Multiple restriction sites can cause difficulties in interpretation
VNTR	10 ⁻² –10 ⁻³	Sex-independent; highly polymorphic markers	Sensitivity is limited by the co-amplification of alleles with different origins; substantial allele size differences can lead to underestimation of the amount of larger alleles; not suitable for allele-specific real-time quantitative PCR
STR	10 ⁻² –5 × 10 ⁻⁴	Sex-independent; highly polymorphic markers; most widely used in routine practice	
SNP	10 ⁻³ –10 ⁻⁵	Sex-independent; high sensitivity	Identification of small differences between alleles is required; which increases assay complexity; SNP loci have limited informativity
INDEL	10 ⁻⁴ –10 ⁻⁵	Sex-independent; high sensitivity; lack of nonspecific amplification	Identification of marker for every patient is needed
Y-QPCR	10 ⁻⁵ –10 ⁻⁶	The most sensitive method	Its power is utilizable only in sex-mismatched cases with female donors

FISH, fluorescence in situ hybridization; RFLP, restriction fragment length polymorphism; VNTR, variable number of tandem repeats; STR, short tandem repeat; SNP, single nucleotide polymorphism; INDEL, short inversion/deletion polymorphism; Y-QPCR, Y-chromosome-specific quantitative polymerase chain reaction.

acute lymphoblastic leukemia who received an allo-BMT from his sister. At the time of post-allo-BMT relapse, karyotyping, fluorescence in situ hybridization, Y chromosome-specific polymerase chain reaction and analysis of the chromosome 17-derived variable number of tandem repeat marker YNZ-22 suggested the presence of DCL. Fortunately, the study also involved molecular cloning and sequencing of the CDRIII region of the immunoglobulin heavy chain gene, which revealed a host origin for the returning disease. It is also worth noting that in almost 30% of published DCL cases, the types of DCL and original disease were identical,⁸ suggesting that some of these patients had recurrent disease rather than DCL.

During the post-allo-BMT period, an efficient strategy for chimerism monitoring is crucial to distinguish relapse from remission and recurrent disease from DCL. The latter issue raises some basic methodological questions: Which technique is the best one? What level of sensitivity is required? Is it necessary to use complex methodologies? Relevant technologies have continued to progress over the last two decades, and a concise overview of the genetic tests frequently used for estimating chimerism is presented in Table 1. A sex-independent, multi-targeted

method is essential for reliable donor-host discrimination to avoid having to use a combination of different techniques. Short tandem repeat (STR) analysis meets these criteria and is the most widely used approach in routine practice. However, several laboratories apply an extensive STR panel at diagnosis but use only one or two discriminating markers during follow-up, thus hampering the correct identification of the cell origin. Concerning the sensitivity, some more complex and laborious methods, such as single nucleotide polymorphism and short inversion/deletion polymorphism arrays, are more sensitive than STR analysis, though this sensitivity is important for detecting impending relapse and not indispensable for substantiating DCL. Instead of very high level of sensitivity, which is one of the most preferred parameters of chimerism analyses, an extensive characterization of the donor sample has to be the main focus of DCL identification because only the demonstration of donor-specific markers at the time of clinical relapse can unambiguously prove the presence of DCL.

Acknowledgements

The author acknowledges the efforts of his colleagues, near and far, whose work has formed the basis of this manuscript.

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