Frontiers of Stem Cell Transplantation Monitoring: Capturing Graft Dynamics through Routine Longitudinal Chimerism Analysis*

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Abstract

Quantitative chimerism testing has become an indispensable tool for following the course and success of allogeneic hematopoietic stem cell transplants. In this paper, we describe the current laboratory approach to quantitative chimerism testing based on an analysis of short tandem repeats, and explain why performing this analysis longitudinally is important and feasible. Longitudinal analysis focuses on relative changes appearing in the course of sequential samples, and as such exploits the ultimate potential of this intrinsically semi-quantitative platform. Such an analysis is more informative than single static values, less likely to be confused with platform artifacts, and is individualized to the particular patient. It is particularly useful with non-myeloablative conditioning, where mixed chimerism is common. When longitudinal chimerism analysis is performed on lineage-specific subpopulations, the sensitivity, specificity and mechanistic implications of the data are augmented. Importantly, longitudinal monitoring is a routinely feasible laboratory option because multiplex STR-PCR kits are available commercially, and modern software can be used to perform computation, reliability testing, and longitudinal tracking in a rapid, easy to use format. The ChimerTrack© application, a shareware program developed in our laboratory for this purpose, produces a report that automatically summarizes and illustrates the quantitative temporal course of the patient's chimeric status. Such a longitudinal perspective enhances the value of quantitative chimerism monitoring for decisions regarding immunomodulatory post-transplant therapy. This information also provides unique insights into the biological dynamics of engraftment underlying the fluctuations in the temporal course of a patient's chimeric status.

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The advent of bone marrow transplantation within the last 30 years created a quiet revolution that is currently mushrooming into the new field of regenerative medicine. The procedure is no longer limited to bone marrow cells, but to any convenient source of stem cells. For severe hematopoietic disorders it may be lifesaving. For many leukemias, aplastic anemias and inborn errors of metabolism it can be curative, an achievement unimaginable a few decades ago.

As one may anticipate, a critical phase of hematopoietic stem cell transplantation is the post-transplantation care: insuring complete engraftment and functionality of the transplanted cells in the host/recipient while minimizing the complications. While the laboratory's role in the pre-transplantation process of identifying a histocompatible donor is well publicized, the equally crucial contributions to post-transplantation monitoring are not so well recognized. The laboratory is responsible for the critical task of assessing the status of the transplanted cells in the new host. This information enables the clinicians to gauge whether engraftment has occurred or whether rejection and relapse are imminent. The usual procedure today is known as chimerism monitoring [1-4].

In antiquity, a number of societies created myths around creatures that were fusions of two different animal forms, referred to as a chimera. The chimeric sphinx – Greek or Egyptian – is a combination of a lion's body and a human head; the Minoan Minotaur from Crete combined a man and a bull. Allogeneic HSCT creates a dynamic cellular chimera involving the transplanted donor cells and the recipient host. For this reason, an accurate assessment of this chimeric state (chimerism, Chm) in the patient's blood or bone marrow provides critical information on the progress of engraftment [1-4]. Hematopoietic chimerism is the most useful parameter for this purpose, and in certain circumstances is the only available basis to make such an assessment. In this short overview, we will consider the process of quantitative chimerism testing, its several forms, and future implications. The longitudinal (sequential) sampling approach to this topic, that we have helped pioneer [4-7], will be stressed. It uniquely allows capturing the dynamic graft-host relationship that must be harnessed by the clinical care team. In this process, longitudinal monitoring also provides a better understanding of the immunobiology of this powerful therapeutic modality.

What is quantitative chimerism analysis?

Monitoring of a hematopoietic chimerism following HSCT entails the identification of the donor's cells in the recipient. It can be done using whole cells or using the DNA from these cells, where some unique feature of the cells or their DNA uniquely discriminates between the donor and recipient. Descriptively, the chimeric status of the patient can be graded as either complete or mixed. A *complete* donor chimerism is said to occur when the patient's original cells/DNA are undetectable in the blood or bone marrow, indicating successful engraftment. In contrast, when

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STR-PCR = short tandem repeats-polymerase chain reaction

HSCT = hematopoietic stem cell transplantation

the donor's cells are undetectable, this implies graft failure. On the other hand, a *mixed* chimerism is an indication that there is an ongoing dynamic relation between the donor's cells and the patient's original cells since both will be demonstrable in the same sample. The state of mixed chimerism in a patient may be stable, increasing, decreasing, or variably fluctuating. We expect that each of these conditions corresponds to a particular graft-host state, although currently we do not understand all aspects of this relationship. If the evaluation for chimerism is performed *qualitatively*, based on HLA-DNA mismatches or other qualitative DNA markers, the results merely indicate the presence of complete or mixed chimerism. In contrast, newer *quantitative* methodologies enable estimating the magnitude of the mixed chimerism in the patient. The emergence of this technology afforded, for the first time, the capability to assess the dynamic aspects of graft status. Quantitative assays are based on estimating the *ratio* between the donor and recipient blood cells, and the results are customarily reported as percent chimerism (%Chm). In practice, this ratio is computed for at least one allelic DNA locus shared by the donor and the patient (recipient). More formally, the particular ratio in common use represents the sum of the donor DNA in alleles at the locus divided by the sum of all allelic DNA at the locus. Solving this ratio provides a value for % *donor* chimerism. Many laboratories report % donor chimerism, because it emphasizes the success or failure of the graft, while other centers concerned with HSCT for blood malignancies prefer % recipient chimerism, which emphasizes the status of the tumor burden.

The implementation of quantitative chimerism monitoring primarily entails one of three different conceptual approaches. One approach involves sampling the patient in response to a specific clinical event, such as change in the patient's medical status or following a therapeutic maneuver. However, the biological implications of such isolated, *absolute* magnitudes of %Chm ("snapshots") are largely unknown [8], so that these values are most reasonably interpreted semi-quantitatively (e.g., low, high). A better utilization of the quantitative data requires testing a patient at fixed intervals post-transplantation (e.g., 30, 100, 200 days), thereby allowing the results to be interpreted in relation to statistical norms for prognosis [9-12]. This application still does not fully exploit the analytic advantage afforded by a quantitative platform, namely, direct comparisons of numerical results between samples. With the advent of such methodologies it became possible, for the first time, to track and compare changes in mixed chimerism, over time, in sequential evaluations. Such *longitudinal* assessments uniquely reveal the progressive kinetics of each patient's mixed chimeric state [4,5,12-15].

Quantitative chimerism testing, today, commonly relies on an assay for short tandem DNA repeats. Although the STR assay was developed for use in forensic criminal identification [17], these DNA markers can be effectively used to distinguish donor from recipient cells in an HSCT patient [1-5,8,9,12-15,18]. Each marker occurs at a characteristic chromosomal location, and represents a short core DNA nucleotide sequence that is repeated in tandem multiple times – hence short tandem repeat. Since a core sequence may be repeated at a given allelic locus from 4 to 50 times, STR markers are considered highly *polymorphic* and thereby useful for human identity applications. STR-based chimerism testing is quantitative because instrumentation exists to estimate the amount of STR-DNA at a marker locus, thereby enabling the computation of the above noted ratio. Another key advantage of the STR platform is that it can be utilized in virtually all donor-recipient combinations, regardless of gender, HLA, or disease type; the only exceptions are identical twins.

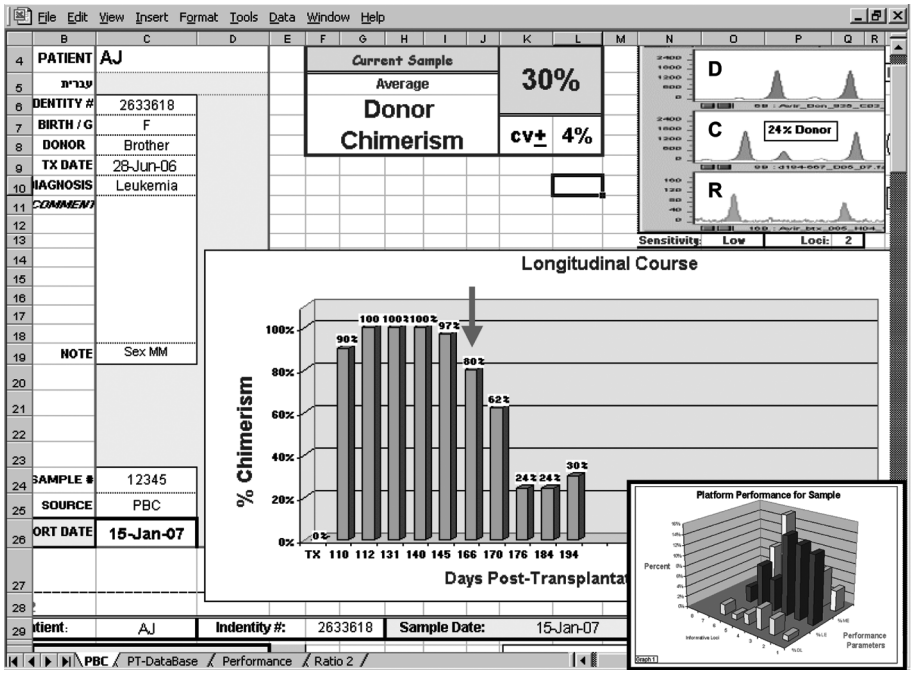

Figure 1. Screen capture from ChimerTrack©, an Excel-based computational-display utility for longitudinal chimerism monitoring. Pictured here is the first of three pages, which is issued to the clinician as the report. It contains areas for patient and sample information (left margin), display of the current sample's numerical results for % Donor Chimerism (top, center), a screen-capture of the allelic configuration of an actual chimeric locus, C, compared to the donor, D, and recipient, R, alleles (top, right), and the graph of the cumulative longitudinal course including the present sample and all previous samples (bottom, right). Arrow in this graph indicates the onset of relapse, represented by a progressive decline in donor chimerism. All the data processing phases of the program operate by a simple copy/paste operation. The inset (lower right), shows the program's graphic display for the reliability assessment parameters that are computed automatically from the same data used to compute % chimerism.

What is longitudinal chimerism analysis?

Longitudinal analysis focuses on relative changes in magnitude and direction appearing in the course of sequential samples, and as such, exploits the ultimate potential of this intrinsically semi-quantitative STR platform [4,5]. Such an analysis is more informative than are single static values, less likely to be confused with platform artifacts, and is individualized to the particular patient. A drop or rise in a particular patient's chimerism levels can be interpreted as a basis for clinical intervention or response to therapy. In the absence of specific tumor markers, sequential analysis of %Chm may offer the only evidence on which sub-clinical relapse of disease can be assessed [Figure 1] [4,5,12-15]. It is particularly useful with non-myeloablative conditioning, where mixed chimerism is common. Such a longitudinal perspective enhances the value of quantitative chimerism monitoring for decisions regarding immunomodulatory post-transplant therapy. This information also provides unique insights into the biological dynamics of engraftment underlying the fluctuations in the temporal course of a patient's chimeric status. In addition to its diagnostic relevance for the individual patient, routine longitudinal chimerism monitoring concomitantly generates data on groups of patients regarding the temporal dynamics of their chimeric state. These data can provide an important resource for clinical investigations on the progressive kinetics of engraftment in relation to disease or treatment variables. Importantly, longitudinal monitoring is a routinely feasible laboratory option because multiplex STR-PCR kits are available commercially, and modern software can be used to perform computation, reliability testing, and longitudinal tracking in a rapid, easy to use format. The ChimerTrack application, a shareware program developed in our laboratory for this purpose [6], produces a report that automatically summarizes and illustrates the quantitative temporal course of the patient's chimeric status. It also performs reliability testing of the chimerism results [7]. This is a critical aspect of the monitoring because the STR measurements used to compute %Chm are only relative, indirect measures of DNA without internal calibration. ChimerTrack provides the analytic tool to perform this step automatically in the process of computing the %Chm.

Table 1. Comparison of advantages of STR-based longitudinal chimerism analysis on peripheral blood samples versus lineage-specific subpopulations

Longitudinal chimerism analysis on PBCs	Longitudinal multi-lineage chimerism analysis
Permits interpreting %Chm in relative terms	Has all of the advantages of PBC-based analysis
Provides sensitivity of approximately 3–5%	Improves sensitivity of assay by several orders of magnitude: detection of < 0.1% recipient cells
Shows graft-host relationship temporally; predicts clinical events	Overcomes masking effect seen in PBC-based chimerism analysis resulting from high levels of engraftment of one fraction (typically donor granulocytes) obscuring the occurrence of poor T cell engraftmen.
Permits individualizing evaluation of chimeric course	Highlights the quantitative temporal relationships between various hematopoietic subpopulations
Facilitates detection of platform errors	Provides insights into the mechanistic implications of the chimeric levels in a patient
Provides more information, e.g., on: Magnitude of change Rate of change Forecasted status	Enables following the results of therapy when the level of T cell engraftment is low, and T cell chimerism is masked in PBC-based analysis
Enables assessing effects of therapy	

What is multi-lineage chimerism analysis?

Up to this point, we have assumed that the nucleated blood cell population in the circulation (peripheral blood cells) and bone marrow was homogeneous, which is clearly not the case [16,19,20]. Although chimerism levels and engraftment status are indeed usually paralleled in all blood cell populations [20], or lineages (e.g., granulocytes, T cells, B cells, natural killer cells), many exceptions occur in specific patients. The dynamics of this variability are not well understood.

Lineage-specific chimerism analysis is the assay of choice, particularly for non-ablative HSCTs, according to a 2001 workshop convened to set chimerism testing standards [19]. This approach focuses on an evaluation of specific cell types, particularly T cells. They provide information often critical for accurately assessing a patient's prognosis and characterizing the events of his clinical course. Additionally, by using LSCA, the sensitivity of the STR-based assay is increased several orders of magnitude compared to PBCs, since you are dealing with highly enriched subset fractions [17]. This approach is also beneficial in some cases of mixed chimerism, where the myeloid chimerism initially may be dominant and thus masks clinically significant changes in other subsets [4,20]. In Table 1, the advantages of STR-based longitudinal chimerism analysis on peripheral blood samples are compared to the results of lineage-specific subpopulation analysis.

A longitudinal approach is complementary to lineage-specific testing for the same reasons it is useful for the evaluation of PBC-based chimerism. Monitoring chimerism longitudinally is applicable whether the evaluation is on a single subset or multiple lineages, concomitantly [4]. In the case of multi-lineage analysis, the complex temporal, directional and magnitude changes in chimeric level are more clearly visualized with a longitudinal approach, thus aiding clinical interpretation of the findings. From a mechanistic perspective, clarifying the interwoven kinetics of different types of effector lymphocytes is also more informative than an assessment of a static data set.

Perspectives for the future

Since quantitative longitudinal chimerism analysis provides a historical record of the engraftment status in the patient, we obviously have a unique opportunity to utilize this individualized data for new, and better modes of monitoring and prognostica-

LSCA = lineage-specific chimerism analysis
PBCs = peripheral blood cells

tion. The ultimate application will be to integrate the dynamic aspects of engraftment status with the functionality of a graft within its new milieu. Preliminary mathematical analysis of the historical data has already suggested a basis for better, and more futuristic monitoring of the post-transplantation condition of the patient [4]. The ultimate goal for chimerism monitoring in the post-HSCT period is to use the data more predicatively to anticipate deterioration in graft status, and initiate early, milder forms of therapy. At the same time, this approach provides a unique opportunity for unique insights into the complex, dynamic relationships being played out between the host and its hematopoietic colonist. A deeper understanding of this relationship can be expected to impact on the development of new pharmacological tools and management strategies leading to an improved outcome for the patient.

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Just as appetite comes by eating, so work brings inspiration, if inspiration is not discernible at the beginning

Igor Stravinsky (1992-1971), Russian-born composer and later American citizen (1945). His father was an opera singer and Stravinsky was a pupil of Rimsky-Korsakov in St. Petersburg. He became famous with the series of ballet scores commissioned by Diaghilev for the Ballets Russes, including *The Firebird, Petrushka*, and *The Rites of Spring*, which was extremely modern in its use of rhythm and dissonance, provoking demonstrations at its premiere and had a strong influence on 20th century music.