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Cell lineage-specific chimerism in post-hematopoietic stem cell transplant patients

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Hematopoietic stem cell transplantation (HSCT) can be an effective method to treat various hematological, oncological, autoimmune and genetic diseases. Clinically relevant data regarding donor chimerism levels in specific cell fractions of post-HSCT patients can be obtained by analysis of patient STR profiles. Adams et al. *show that this type of analysis is enhanced by rapid pre-sorting with MACS® MicroBeads.*

Cell lineage-specific chimerism in post-hematopoietic stem cell transplant patients.

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Introduction

The use of low-intensity conditioning (LIC) with hematopoietic stem cell transplantation (HSCT) for higher-risk patients at our center (both for hematological malignancy and congenital immunodeficiencies) has increased over recent years[1]. The use of LIC HSCT often results in mixed chimerism of donor and recipient cells existing in a state of host and graft tolerance. However, what has been unclear until now is the relative contribution of donor cells to the different T, B and myeloid cell lineages. This has important implications for certain congenital immunodeficiencies where engraftment of donor cells in multiple cell lineages may be required for disease correction long-term or, conversely, for certain immunodeficiencies where donor engraftment in a particular lineage is sufficient for disease correction. We have introduced laboratory techniques to provide detailed information on donor engraftment levels in various cell lineages.

Materials and Methods

Whole blood CD3, CD15 and CD19 MicroBeads were used with the autoMACS® Separator (Miltenyi Biotec) to isolate T cells, neutrophils, and B cells, respectively, from whole blood samples taken from post-HSCT patients. Typically, 50 µL CD15 Whole Blood MicroBeads were used with 1 mL of whole blood, while 100 µL CD3 and CD19 Whole Blood MicroBeads were used with 2 mL whole blood. DNA was extracted from pre-HSCT, post-HSCT and donor samples using the QIAamp DNA Blood Mini Kit (Qiagen UK Ltd.). Multiplex PCR amplification of eight fluorescence-labeled short tandem repeats (STRs) and a sex discrimination marker (amelogenin) was performed using the PowerPlex 1.2 System (Promega UK Ltd).[2] Amplified STRs were genotyped on a MegaBACE sequencer (Amersham Biosciences UK Ltd.) and analyzed using Genetic Profiler software.

Patient	Disease	Type of HSCT	PB/WB	CD3+	CD15+	CD19+
P1	ADA-SCID	NC, MFD	38%	100%	0%	0%
P2	X-SCID	NC, Haplo	51%	100%	0%	2%
P3	Undefined SCID	C, MUD	16%	44%	17%	11%
P4	Undefined SCID	C, MUD	17%	77%	5%	0%
P5	WAS	C, MUD	30%	84%	4%	63%
P6	Neutrophil disorder	C, MUD	95%	22%	100%	63%

Table 1 Examples of cell lineage-specific chimerism analyses for congenital immunodeficiencies
NC = no conditioning, C = conditioning,
MFD = matched family donor, haplo = haplo-identical donor,
MUD = matched unrelated donor.

Results

To date we have used MACS MicroBeads-based cell separation, followed by fluorescent detection of multiplex PCR-amplified STRs to monitor the engraftment status in 30 patients post HSCT. Many of these patients were transplanted for congenital immunodeficiencies using LIC and have subsequent mixed chimerism.

The relative contribution of donor cells to the different T, B and myeloid cell lineages may have important implications for certain congenital immunodeficiencies where engraftment of donor cells in multiple cell lineages may be required for disease correction long term. Examples of the use of cell-separated chimerism analysis in our center are provided in table 1. In P1 and P2, who received unconditioned HSCT, there is no evidence of B cell engraftment (P1 STR profile shown in figure 1). In P3 and P4, who had undefined SCID, conditioned HSCT have led to functional T cell recovery but decreased engraftment in other cell lineages. However, this is sufficient for correction of the clinical phenotype. In P5, who had Wiskott-Aldrich Syndrome (WAS), decreased engraftment in the myeloid cells may have functional consequences given the multi-lineage defects in WAS. In P6 – who had a neutrophil disorder – conditioned HSCT has led to total engraftment in the neutrophil fraction but decreased engraftment in other cell lineages (figure 2).

We have also used magnetic bead separation to isolate other cell fractions specifically related to particular diseases. For example, we used the BDCA-1 Dendritic Cell Isolation Kit (Miltenyi Biotec) to isolate dendritic cells from whole blood from post-HSCT patients transplanted to treat Langerhans Cell Histiocytosis (LCH).

Discussion

We have found that the use of multiplex amplification of STR markers with fluorescence detection to monitor post-HSCT donor cell engraftment allows for rapid identification of informative markers and also enables the calculation of mean donor values for several STRs, increasing the accuracy and reproducibility of the results.

Monitoring chimerism levels in whole blood samples from post-HSCT patients has limitations regarding clinical interpretation. To further understand the donor engraftment process and to provide clinically relevant data it is often necessary to analyze donor chimerism levels in specific cell fractions. This can be particularly important in the setting of LIC HSCT where mixed chimerism in the whole blood is common. Rapid MACS MicroBeads sorting of specific cell fractions has facilitated this process.

References

1. Gaspar, H. B. *et al.* (2002) Recent Results Cancer Res. 159:134–42.
2. Thiede, C. *et al.* (2004) Leukemia 18: 248–254.

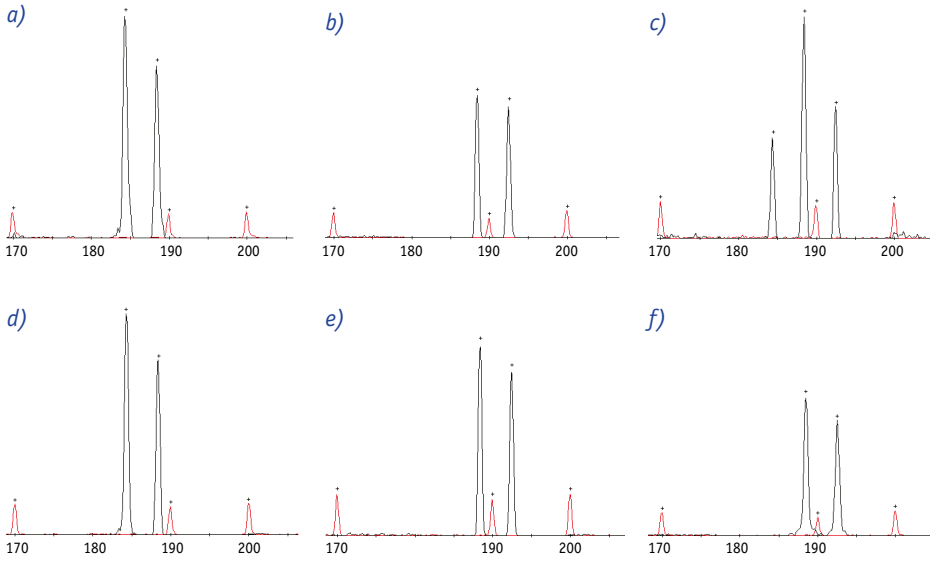

Figure 1 Patient 1 profile for one STR at 12 months post HSCT. Red peaks indicate size marker (size given in base pairs along x-axis). Black peaks represent a heterozygous STR in; a) donor; b) pre HSCT; c) whole blood post HSCT; d) $CD3^+$ T cells post HSCT; e) $CD15^+$ neutrophils post HSCT; f) $CD19^+$ B cells post HSCT samples. Mixed chimerism seen in whole blood (38% donor), full donor engraftment seen in $CD3^+$ T cells, no donor engraftment seen in $CD15^+$ neutrophils or $CD19^+$ B cells.

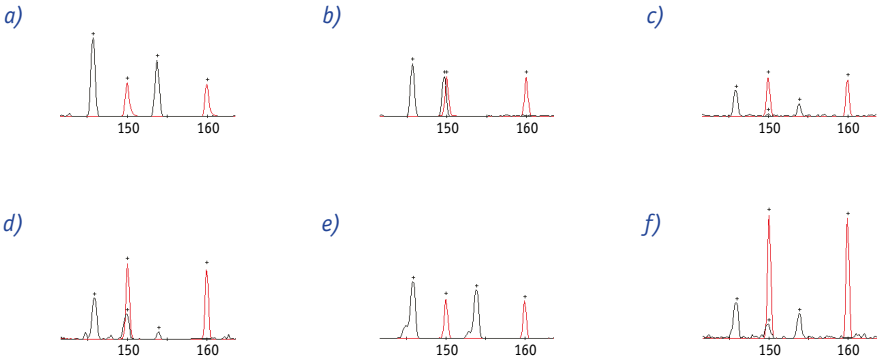

Figure 2 Patient 6 profile for one STR at 6 months post HSCT. Red peaks indicate size marker (size given in base pairs along x-axis). Black peaks represent a heterozygous STR in; a) donor; b) pre HSCT; c) whole blood post HSCT; d) $CD3^+$ T cells post HSCT; e) $CD15^+$ neutrophils post HSCT; f) $CD19^+$ B cells post HSCT samples. Mixed donor chimerism seen in whole blood (95% donor), $CD3^+$ T cells (22% donor), $CD19^+$ B cells (63% donor). Full donor engraftment is seen in $CD15^+$ neutrophils.