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# Ultra-Sensitive Droplet Digital PCR for the Assessment of Microchimerism in Cellular Therapies

David Kliman [1], Gloria Castellano-Gonzalez [1], Barbara Withers [1,2], Janine Street [1], Elizabeth Tegg [2,3], Oksana Mirochnik [3], Joey Lai [1], Leighton Clancy [1,3,4], David Gottlieb [1,2,4,5], Emily Blyth [1,2,4,5,*]

[1] *Westmead Institute of Medical Research, University of Sydney, Westmead, Australia*
[2] *Sydney Medical School, University of Sydney, Camperdown, Australia*
[3] *Institute of Clinical Pathology and Medical Research (ICPMR), NSW Health Pathology, Westmead, Australia*
[4] *Sydney Cellular Therapies Laboratory, Westmead, Australia*
[5] *Blood and Marrow Transplant Unit, Department of Haematology, Westmead Hospital, Westmead, Australia*

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Current techniques to assess chimerism after hematopoietic stem cell transplantation (HSCT) are limited in both sensitivity and precision. These drawbacks are problematic in the context of cellular therapies that frequently result in microchimerism (donor chimerism <1%). We have developed a highly sensitive droplet digital PCR (ddPCR) assay using commercially available regents with good performance throughout the range of clinically relevant chimerism measurements, including microchimerism. We tested the assay using spiked samples of known donor-recipient ratios and in clinical samples from HSCT recipients and patients enrolled on clinical trials of microtransplantation and third-party virus-specific T cells (VSTs). The levels of detection and quantification of the assay were .008% and .023%, with high levels of precision with samples of DNA content ranging from 1 to 300 ng DNA. From the panel of 29 insertion-deletion probes multiple informative markers were found for each of 43 HSCT donor-recipient pairs. In the case of third-party cellular therapies in which there were 3 DNA contributors (recipient, HSCT donor, and T-cell donor), a marker to detect the cellular product in a background of recipient and donor cells was available for 11 of 12 cases (92%). Chimerism by ddPCR was able to quantify chimerism in HSCT recipients and comparison against standard STR analysis in 8 HSCT patients demonstrated similar results, with the advantage of fast turnaround time. Persistence of donor microchimerism in patients undergoing microtransplantation for acute myeloid leukemia was detectable for up to 57 days in peripheral blood and bone marrow. The presence of microtransplant product DNA in bone marrow T cells after cell sorting was seen in the 1 patient tested. In patients receiving third-party VSTs for treatment of refractory viral infections, VST donor DNA was detected at low levels in 7 of 9 cases. ddPCR offers advantages over currently available methods for assessment of chimerism in standard HSCT and cellular therapies.


## INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) provides a potentially curative therapy for a number of malignant and nonmalignant hematological conditions. This treatment relies on the establishment of donor hematopoiesis in the recipient but can result in prolonged periods of mixed donor and recipient chimerism. When HSCT is performed for malignant disease, prolonged persistence or recurrence of mixed chimerism is generally considered undesirable as it is associated with disease recurrence. The use of cellular infusions is becoming more commonplace in transplant practice. These include third-party donor antigen-specific T-cell infusions for treatment of infections and nonengrafting adoptive cellular therapies such as natural killer (NK) cell infusion [1,2] and microtransplantantation in acute myelogenous leukemia (AML) [2-8]. Depending on the clinical situation, highly sensitive detection of states of mixed chimerism may assist in the detection of early relapse of malignancy or could predict therapeutic benefit following cellular therapies.

Sensitive methods of quantifying chimerism with precision and rapid turnaround are needed. Currently, short tandem

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* Correspondence and reprint requests: Emily Blyth, Med(Hons), FRACP, FRCPA, PhD, Blood and Marrow Transplant Unit, Department of Haematology, Westmead Hospital, Sydney, NSW 2145, Australia.
*E-mail address:* emily.blyth@sydney.edu.au (E. Blyth).

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repeat (STR)–based methods are utilized in many clinical transplant laboratories. When optimized, this assay has a sensitivity of .8% to 1.6% and is not suitable for assessment of chimerism in the micro range (<1%) [9]. Quantitative PCR (qPCR) methods [10] have inherent technical complexity limiting their uptake in clinical laboratories. Droplet digital PCR (ddPCR) methods that show improved sensitivity and precision compared with previous methods have been reported [11-14], but the need to establish a set of informative targets in house is beyond the scope of most clinical laboratories. To address this, we sought to validate a sensitive and precise ddPCR chimerism assay by using commercially available reagents for use in a general clinical transplant setting.

## METHODS

The study was conducted with approval from Western Sydney Local Health District Ethics Committee.

### *Study Participants*

Blood from otherwise healthy individuals undergoing venesection for hemochromatosis was used for validation assays. Three sets of clinical samples were assessed (see Figure 1). First, peripheral blood samples obtained for chimerism monitoring from patients undergoing HSCT for AML from matched related or cord blood donors. This sample set included patients with AML who underwent reduced intensity conditioning and were known to have had mixed donor-recipient chimerism through the post-transplant course. Second, peripheral blood from HSCT recipients who received $2 \times 10^7$ cells/$m^2$ virus-specific T cells (VSTs) from third-party partially HLA-mismatched donors on a clinical trial (R3ACT trial; NCT02779439). This sample set included triplets of the recipient, original transplant donor, and the third-party VST donor. Third, peripheral blood and marrow samples from patients with AML undergoing microtransplantation with partially HLA-matched unrelated donors after consolidation chemotherapy on a clinical trial (SCRAMBLE trial; NCT02189824). These patients received 2 g/$m^2$ of cytarabine twice a day on days 1 to 3 of each of 3 consolidation cycles with $0.5 \times 10^8$ CD3 cells/kg on day 5 without the use of immune suppression. This sample set included pairs of recipient and microtransplant donor. Clinical results of the clinical trials are reported separately [15,16].

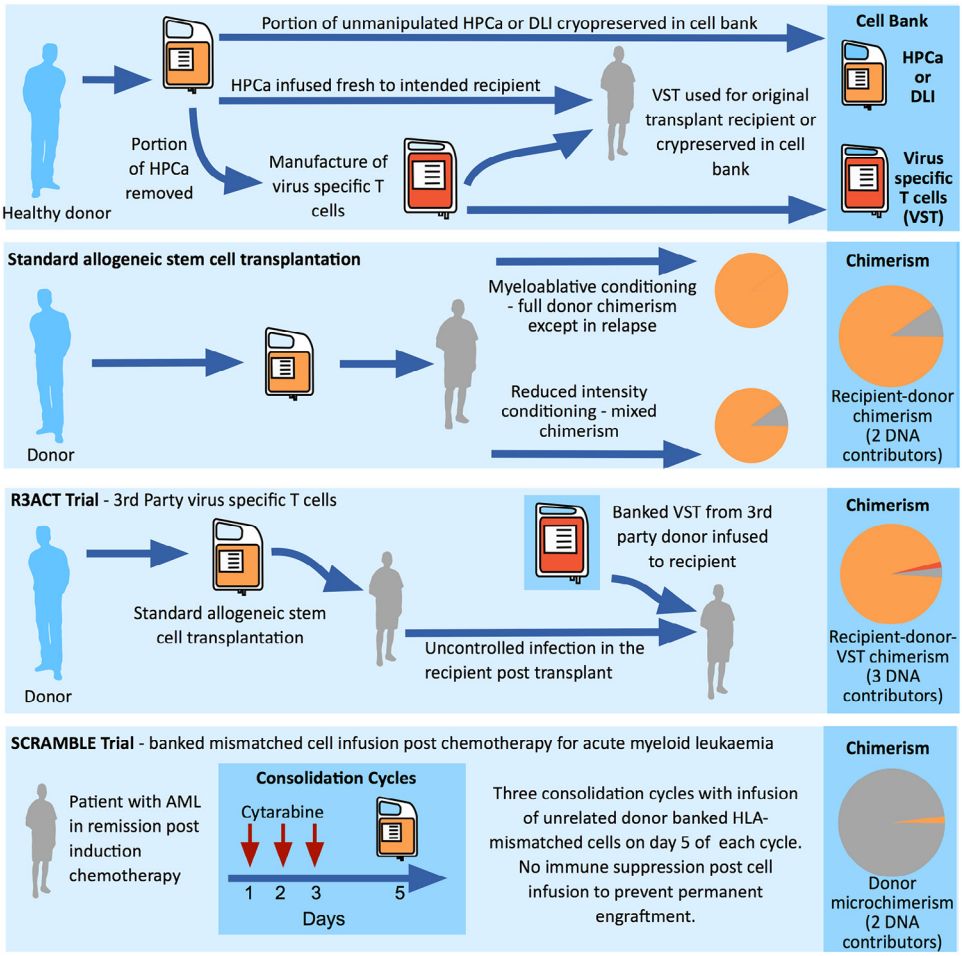


**Figure 1.** Clinical scenarios of samples used for this study and expected chimerism.
**Panel 1.** Samples obtained from healthy HSCT donors, recipients, and 2 cell banks were used in this study. Hematopoietic progenitor cell apheresis (HPCa) or donor lymphocyte infusion (DLI) was directly administered to transplant recipients and additional material cryopreserved for later use. Cryopreserved HPCa or DLI was entered into the cell bank when no longer needed by the original transplant recipient (SCRAMBLE trial cell bank). A portion of the fresh HPCa or DLI was used for manufacture of VSTs for the R3ACT trial cell bank.
**Panel 2.** Standard hematopoietic stem cell transplantation resulting in mixed recipient-donor chimerism of variable degree depending on conditioning, post-transplant immune suppression and disease status.
**Panel 3.** Microchimerism of third-party donor–derived VST (R3ACT trial). Patients who developed viral reactivation that did not respond to first line antiviral therapy were administered third-party VSTs. This resulted in mixed chimerism with 3 DNA contributors (recipient, stem cell donor, and third-party T-cell donor).
**Panel 4**. Microchimerism from microtransplantation (SCRAMBLE trial). Patients with AML in remission underwent 3 consolidation cycles comprising intermediate dose cytarabine followed by infusion of partially HLA-matched unrelated donor cells (HPCa or DLI) from the cell bank. No immune suppression was used and long-term engraftment was not expected.