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The immune reconstitution of the skin following sex-mismatched allogeneic haematopoietic stem cell transplant: a prospective case series utilizing fluorescence *in situ* hybridization and immunohistochemistry

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Dear Editor, Graft-versus-host disease (GVHD) is a common, morbid and potentially fatal complication of allogeneic haematopoietic stem cell transplantation (aHSCT).^{1–3} The exact timing of the arrival of donor-derived immune cells and their profile in the skin have not been well described in humans.^{4–6} Fluorescence *in situ* hybridization (FISH) is a technique that has been used to distinguish host-versus-donor-derived immune cells in patients who have undergone sex-mismatched aHSCT.⁷

To investigate the changing immune profile of the skin, we designed a prospective analysis of skin biopsy specimens obtained from seven patients undergoing sex-mismatched aHSCT. FISH (X and Y chromosome; CEP X SpectrumOrange/ Y SpectrumGreen DNA Probe Kit, Vysis/Abbot Molecular, Des Plaines, IL, U.S.A.) and immunohistochemistry studies (CD1a, CD3, CD4, CD8, CD20 and CD56) were performed on skin biopsy samples obtained immediately prior to transplant, on day 14 post-transplant, at engraftment and in the setting of acute GVHD (aGVHD), from both normal and lesional skin. Tissue specimens were serially sectioned per our protocol, allowing for comparison of each immunohistochemistry stained slide with an adjacent FISH slide in parallel in order to determine cell origin (i.e. host or donor). Counts of immunohistochemically labelled cells and FISH-positive donor cells in the epidermis and along the dermoepidermal junction were performed manually on three serial sections, two optical fields each at 100×. Dermal cells were not included in the analysis.

Three of the seven patients were excluded: one because of engraftment failure, one because of treatment of GVHD prior to sampling and one because of development of a drug eruption.

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The four remaining patients all developed features of aGVHD (median onset: day 216). No patient developed clinical evidence of chronic GVHD during the study. There was no correlation between donor chimerism, full CD3+ chimerism, the development of GVHD and the number of donor cells visible in the skin. Donor cells approximated the epidermis in aGVHD-affected skin as early as day 23 (Fig. 1a). Donor cells were absent prior to transplant, on day 14 post-transplant, at engraftment (unless aGVHD coincided with engraftment) and in aGVHD-unaffected skin. aGVHD was mediated primarily by CD3+CD8+ T cells (Fig. 1b). Nondendritic CD4+ cells were too low in number to determine accurately their role in the post-transplant setting. Intraepidermal CD1a+ Langerhans cells were depleted in day 14 biopsies and were lowest in those receiving fully myeloablative transplant/total body irradiation; counts did not differ with the presence of aGVHD (Fig. 1b). CD56 and CD20 staining was negligible in all specimens.

Based on these results, we observe that donor-derived cells are present and CD8+ cells are increased in the epidermis at the time of aGVHD diagnosis. In the post-transplant setting, the FISH technique is a useful adjunctive test to highlight immune cell population dynamics in the skin of sex-mismatched transplant recipients. Limitations of our study include a small sample size and lack of long-term clinical and histological follow-up. Further prospective studies of the skin after aHSCT and in GVHD using a larger cohort and including subsequent time points with co-labelling of FISH and immunofluorescence techniques could elaborate on these results.

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Fig 1.

(a) Female patient with evidence of male donor cells in lesional but not unaffected skin at the time of acute graft-versus-host disease (GVHD) onset (fluorescence *in situ* hybridization, original magnification $\times 200$, orange, X chromosome; green, Y chromosome; 4',6-diamidino-2-phenylindole nuclear stain). S. Corneum, stratum corneum. (b) The immune profile of the skin following allogeneic haematopoietic stem cell transplantation. Cell counts represent the mean ($\pm$ standard error) number of positively labelled cells for each antibody at distinct time points in the study (cell counts performed on three serial sections, two optical fields each, at $100\times$).