

### The origin of epithelial neoplasms after allogeneic stem cell transplantation

**We analyzed five women, who have developed epithelial neoplasms after sex-mismatched stem cell transplants. Using *in situ* hybridization for sex chromosome-specific DNA probes and immunohistochemistry we identified the origin of the tumor cells. We conclude that none of the non-hematologic malignancies was of donor origin.**

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Besides hematopoietic stem cells, bone marrow contains mesenchymal stem cells that differentiate into mature cells of mesenchymal tissues such as muscle, bone, and cartilage. Over the years bone marrow-derived stem cells have become of great interest for researchers, mainly because of the high degree of plasticity of these cells.<sup>1</sup> It has been suggested that bone marrow-derived cells are frequently recruited to sites of tissue injury to replace damaged cells. Whether tissue injury is necessary to recruit stem cells remains unclear. The existing theory about the stem cell origin of tissues has led to a closely related theory that cancer could also be of stem cell origin, as reviewed by Sell.<sup>2</sup> Whether bone marrow-derived cells could actually be a potential source of malignancy, was recently investigated.<sup>3</sup> Using the *Helicobacter felis*/C57BL/6 mouse model for gastric cancer, in which mice are irradiated before infection and transplanted with bone marrow cells that bear a genetically engineered marker to distinguish donor cells from recipient cells, they showed that chronic *Helicobacter* infection induces repopulation of the stomach with bone marrow derived cells and that these cells eventually progress into intra-epithelial cancer. These findings led us to question whether non-hematologic malignancies that have developed in patients after stem cell transplantation (SCT) are of donor origin. We analyzed tumor tissues from five women, who had been transplanted with T-cell depleted

marrow from an HLA-identical brother, and who had developed non-hematologic neoplasia without severe chronic graft-versus-host disease (Table 1). Clinical data and the patients' material were obtained from the archives of the Departments of Haematology and Pathology of Radboud University Nijmegen Medical Centre. In total 150 women have undergone sex-mismatched SCT, and the incidence of secondary malignancies in this group was similar to that in the group receiving gender-matched grafts. The number and type of secondary malignancies that occurred in our series were in line with those reported in the literature.<sup>4</sup>

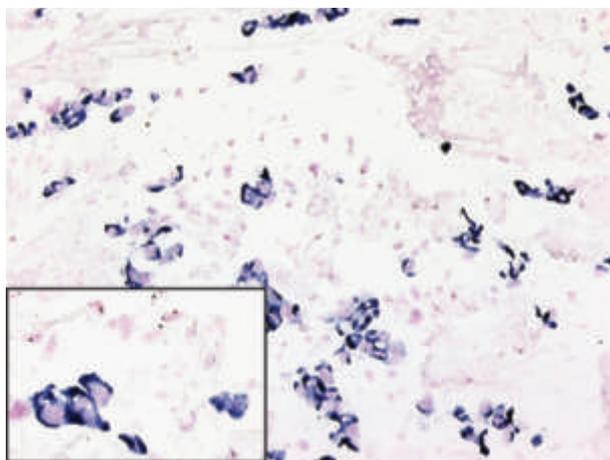
We combined immunohistochemistry and *in situ* hybridization (ISH) to analyze the origin of the tumor cells. To identify tumor cells, tissue sections were incubated with monoclonal antibody against CAM5,<sup>2</sup> and power-alkaline phosphatase, respectively. Visualization was then performed with 4-nitro blue tetrazolium chloride (NBT)-5-bromo-4-chloro-3-indolyl phosphate (BCIP) solution. For ISH the satellite III DNA probe for chromosome Y (DYZ1) was used to identify the presence of donor cells in the tumors of transplant recipients. Probe and target DNA were denatured and hybridization was performed overnight at 37°C. The probe was detected by mouse anti-biotin (Dako, Glostrup, Denmark), biotin-labeled horse anti-mouse, and avidin-biotin-labeled peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories Inc., Burlingame, CA, USA). Visualization of the DNA probe was performed with 0.5 mg/mL 3,3-diaminobenzidine tetra hydrochloride. Slides were counterstained with nuclear fast red, dehydrated. Prior to the previously described double staining, ISH was performed for chromosomes Y and X.

The analyzed lesions developed between 1.5 and 10 years after transplantation, and consisted of pre-malignant and fully developed cancers of various grades of malignancy. In all cases donor inflammatory cells, mainly lymphocytes (based on morphology), were present, as expected. However, all five (pre-)neoplasms were of recipient origin since in all cases the tumor cells did not contain chromosome Y (Figure 1). In addition, the tumor cells showed diploidy and polyploidy (in two patients with a ductal carcinoma *in situ*) for chromosome X (*data not shown*). In agreement with previous research,<sup>5</sup> our data demonstrate the presence of bone marrow derived

**Table 1.** Female patients with non-hematological malignancies after sex-mismatched stem cell transplantation (SCT).

Patient's sex	Patient's age at SCT	Indication for SCT <sup>1</sup>	Donor-type <sup>2</sup>	Conditioning for SCT <sup>3</sup>	CD3 <sup>+</sup> cells left in the graft ( $\times 10^6/\text{kg}$ )	GVHD acute/chronic <sup>4</sup>	Interval SCT-diagnosis carcinoma (years)	Histological diagnosis
F	47	NHL	HLA-id	TBI plus CPM	0.74	0/0	1.5	Moderately differentiated invasive ductal carcinoma
F	52	MM	HLA-id	Ida plus TBI plus CPM	0.74	I / L	5	Well differentiated invasive ductal carcinoma
F	51	RAEB-t	HLA-id	Ida plus TBI plus CPM	0.72	I / 0	8.5	Poorly differentiated ductal carcinoma <i>in situ</i>
F	56	MM	HLA-id	TBI plus CPM	0.72	II / 0	3	Basocellular carcinoma
F	54	RA	HLA-id	Ida plus TBI plus CPM	0.70	I / L	10	Lobular carcinoma in situ

<sup>1</sup>NHL: Non-Hodgkin's lymphoma; MM: multiple myeloma; RAEB-t: refractory anemia with an excess of blasts in transformation; RA: refractory anemia; <sup>2</sup>HLA-id: HLA-identical brother; <sup>3</sup>TBI: total body irradiation 9 Gy; CPM: cyclophosphamide 120 mg/kg; Ida: idarubicin 42 mg/m<sup>2</sup>; <sup>4</sup>L chronic GVHD: limited chronic graft-versus-host disease.



**Figure 1.** Invasive ductal carcinoma without bone marrow-derived tumor cells. Immunohistochemical staining of tumor tissue for CAM 5.2 (purple) with *in situ* hybridization for Y chromosome-specific DNA probe (brown dots), counterstained by nuclear fast red. The tumor sample was taken from a 48-years/old female patient, 1.5 years after a stem cell transplant. Y chromosome positive cells were present, but no double positivity for both CAM 5.2 and Y chromosome was detected. Magnification is 200x, and 400x (insert).

endothelial cells. The endothelium within the tumor showed mixed chimerism, which is at least partially the result of neo-angiogenesis. In contrast, other studies have repeatedly shown that post-transplant lymphoproliferative disorders after SCT are mostly derived from donor cells.<sup>6,7</sup> This was expected since SCT can fully replace the hematopoietic system, including lymphopoiesis. Solid tumors arise after SCT even more often,<sup>8</sup> but until now studies into the origin of the tumor cells have not been reported.

The present findings show that none of the epithelial malignancies after SCT was of donor origin. This is in contrast with the experimental model of Houghton and Wang.<sup>3</sup> The major difference is that the *Helicobacter*-associated gastric cancer in their model arises after continued inflammation and tissue repair. The tumors we describe

are not related to chronic inflammation and no such cases were present in our relatively large series with a median follow-up of 10 years. Of course we cannot exclude the rare occurrence of donor-derived cancers, but the mechanism by which most transplantation-associated cancers arise might be different from that in an experimental model. We conclude that the investigated non-hematologic malignancies are derived from recipient rather than donor epithelial cells.

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