

## LETTER TO THE EDITOR

# Heterogeneous loss of the Y chromosome in leukocyte lineages of donor origin after stem cell transplantation

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Loss of the Y chromosome has been described in patients with hematological malignancies representing a disease-associated chromosome marker.<sup>1,2</sup> However, several studies have shown that loss of the Y chromosome with a resulting 45,X,-Y karyotype from bone marrow (BM) and peripheral blood (PB) cells of elderly males is relatively common (c.a. 8% of males older than 60 years) and in most cases must be interpreted as a normal age-related phenomenon with no pathological consequence.<sup>1,3</sup>

Advances of allogeneic stem cell transplantation (SCT), such as reduced intensity conditioning or haploidentical transplant settings, have favored the use of patients and/or donors of increasing age. Therefore, the chance to find healthy donors with loss of the Y chromosome is also increased.

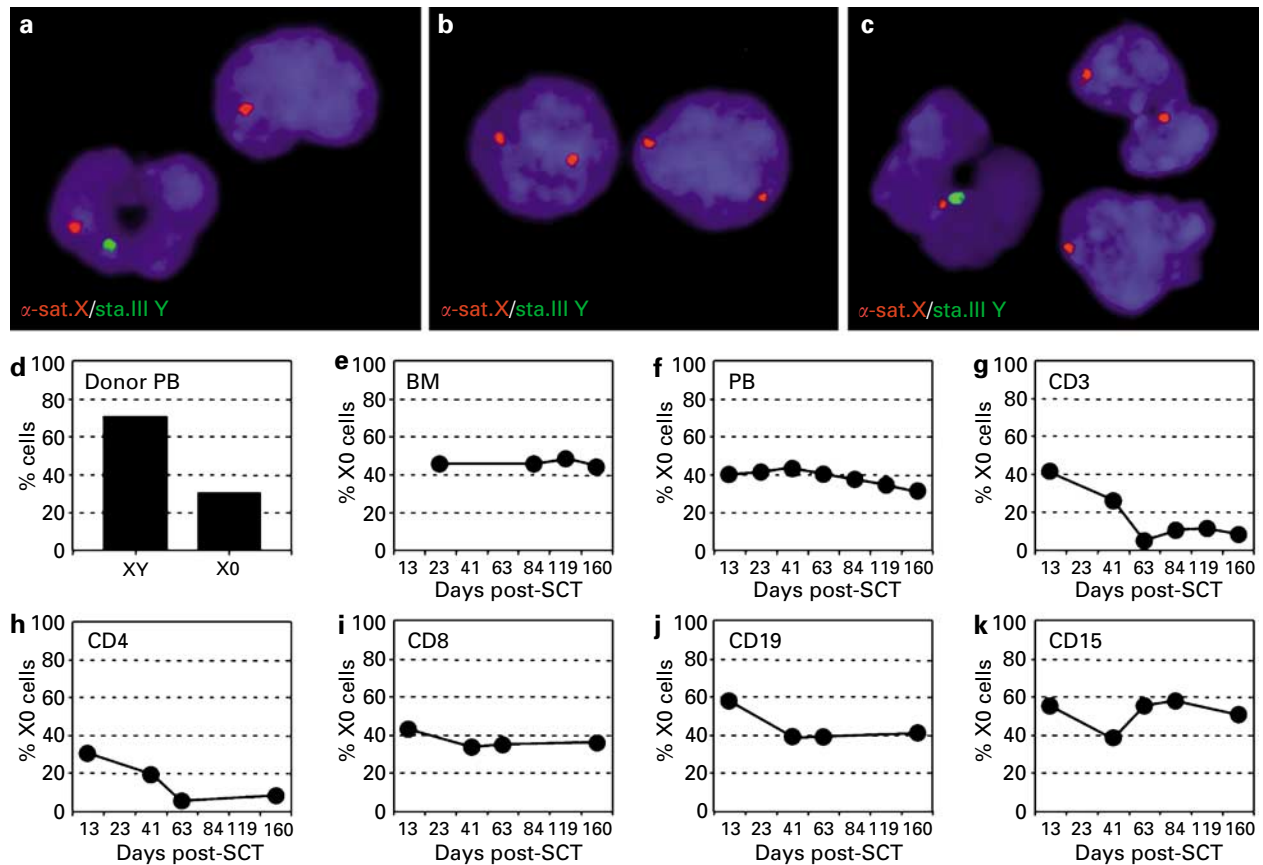
The objective of the present study was to evaluate the influence of the loss of the Y chromosome in a 56 years old male haploidentical donor on the outcome of SCT.

The recipient was a 33-year old female with acute myeloid leukemia (AML) in relapse after autologous SCT who underwent allogeneic SCT from a non HLA-identical family donor (NIFD, father). The conditioning regimen for the allogeneic SCT consisted in thiotepea, fludarabine, melphalan and antithymocyte globulin. The G-CSF mobilized PB graft was T-cell depleted by CD34+ immunomagnetic selection (CliniMACS, Miltenyi Biotec) to contain 7.3 CD34+ and 0.092 CD3+ cells  $\times 10^6$ /kg. The patient received immunosuppressive therapy with cyclosporine A (CsA, 3 mg/kg/day  $\times$  days -1 to +27) as graft-versus-host disease (GVHD) prophylaxis and high dose of intravenous steroids (from day +60) for the treatment of neuropathic toxicity. The patient died from sepsis on day +201 post-SCT.

Chimerism was quantified by fluorescent *in situ* hybridization (FISH) for the sex chromosomes (XY-FISH) on routine PB (Figure 1) and BM smears, as well as on leukocyte lineages purified using immunomagnetic technology (AutoMACS, Miltenyi Biotec): T cells (CD3+), both helper (CD4+) and cytotoxic (CD8+), B cells (CD19+) and myeloid cells (CD15+).<sup>4</sup>

XY-FISH analysis of PB leukocytes from the donor showed the following results: nuclear fluorescence *in situ* hybridization (nuc ish) (DXZ1, DYZ3)x1 [352]/(DXZ1x1, DYZ3x0) [148]. Therefore, 30% of the cells showed loss of the Y chromosome (X,-Y; Figure 1a), and the remaining

70% showed both male sex chromosomes (XY cells). Analysis of recipient PB cells showed nuc ish (DXZ1x2) [500]. All cells from the recipient showed two X chromosomes (XX cells; Figure 1b). Therefore, in post-SCT chimerism studies both XY and X,-Y cells (Figure 1a) were considered to be from the donor and XX cells (Figure 1b) were considered to be residual cells from the recipient. The patient showed complete chimerism (only XY and X,-Y cells) in all samples analyzed post SCT except for the PB sample obtained on day +13 post-SCT (2% XX cells; Figure 1c). The XY/X,-Y cell ratio showed different values and evolution, throughout the post-SCT period, in the different sample types analyzed (Figure 1d–k). The XY/X,-Y cell ratio showed values around 60/40 and did not show significant changes during all post-SCT period in BM, cytotoxic T lymphocytes (CD8+), B lymphocytes (CD19+) and myeloid cells (CD15+). However, it varied from 60/40 to 70/30 in PB at the expense of T lymphocytes (CD3+), mainly helper T lymphocytes (CD4+), in which the XY/X,-Y cell ratio varied from 60/40 and 70/30, respectively, to 95/5. This relative disappearance of X,-Y cells (mainly CD4+ T cells) would suggest that the apparent physiological loss of the Y chromosome seen in males of advanced age could have pathological consequences under special circumstances such as immunologic stress or immunosuppression. In this context, one could speculate that X,-Y cells share similarities with the cells from patients with Turner syndrome (45,X). In fact, it has been shown in Turner syndrome patients show various immunological disorders.<sup>5–7</sup> Moreover, monosomy X in T cells would be related to an increased spontaneous as well as death receptor-mediated apoptosis.<sup>8</sup> In addition, monosomy X T cells and monocytes of these patients showed a decreased sensitivity to insulin-like growth factor I (IGF-1) and growth hormone, and therefore a decreased monocyte-stimulated T lymphocyte proliferation.<sup>9</sup> On the other hand, studies aimed to identify specific genes responsible for the Turner syndrome phenotype suggest that many characteristic features are caused by haploinsufficiency of specific genes for which a diploid dosage would be required for their normal function.<sup>10,11</sup> Although, to our knowledge, no genes have been specifically involved in the mentioned T-cell deficiency, this hypothesis could account for the immune alterations observed in these patients. Within this scenario, a number of studies have described the genes associated with the human Y chromosome and efforts to understand their biological functions are being made.<sup>12,13</sup> Candidate genes to have a role in the proliferation and/or maturation of such cells would be cytokine receptors, such as the interleukin 3 receptor.<sup>14</sup>



**Figure 1** (a) XY-FISH performed on a PB smear from the SCT donor in which the loss of the Y chromosome (green signal) can be observed in 30% of the cells. (b) All cells from the recipient before SCT showed a typical XY-FISH pattern of female cells (XX, two red signals). (c) Mixed chimerism sample post-SCT in which residual cells from the patient (XX) as well as cells from the donor with and without Y chromosome (XY and X,-Y) are observed. (d) XY/X,-Y ratio in the male donor. (e-k) Evolution of the proportion of XY and X,-Y cells all along post-SCT period in different samples studied.

It has been postulated that an age-related increase of X,-Y cells could result in two ways,<sup>1</sup> it could be due to the cumulative loss of the Y chromosome from individual cells through errors of cell division, or it could arise from a single X,-Y cell and a gradual replacement of the XY cells. In the case reported here, the relative increase of XY T cells (mostly CD4+ cells) during the post-SCT period could be either due to a reduced rate of generation/maturation as well as to an increased rate of elimination of X,-Y T cells. In this context, it is possible that X,-Y lymphocytes showed increased sensitivity, compared to normal XY cells, to the high dose of immunosuppressive drugs, through as yet unknown mechanisms, that the patient received during the post SCT period. In any case, regardless of the ultimate cause for the loss of X,-Y T-cells (mainly CD4+ T cells) observed after SCT in the patient reported here, it could have compromised the immune capacity of the patient, who in fact had various infectious complications and died from sepsis on day +201 post-SCT.

In conclusion, male donors, older than 50 years, should be studied by XY-FISH on PB samples in order to rule out the loss of the Y chromosome. If this is detected, the follow-up of the recipient patients, should pay special attention on their immune recovery, in order to gain insight

into its implication in the outcome of SCT, and eventually validate or censure the use of such donors.

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