Published in final edited form as:

Biol Blood Marrow Transplant. 2008 October ; 14(10): 1172–1179. doi:10.1016/j.bbmt.2008.08.003.

Persistence of human parvovirus B19 in multipotent mesenchymal stromal cells expressing the erythrocyte P antigen: implications for transplantation

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Abstract

Multipotent mesenchymal stromal cells (MSC) are used to improve the outcome of hematopoietic stem cell transplantation and in regenerative medicine. However, MSC may harbor persistent

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viruses that may compromise their clinical benefit. Retrospectively screened, 1 of 20 MSC from healthy donors contained parvovirus B19 (B19) DNA. We found that MSC express the B19 receptor (the globoside P antigen) and a co-receptor (Ku 80), and can transmit B19 to bone marrow cells *in vitro*, suggesting that the virus can persist in the marrow stroma of healthy individuals. Two stem cell transplant patients received the B19 positive MSC as treatment for graft-versus-host disease. Neither developed viremia nor symptomatic B19 infection. These results demonstrate for the first time that persistent B19 in MSC can infect hematopoietic cells and underscore the importance of monitoring B19 transmission by MSC products.

Keywords

transplantation; hematopoietic; cell therapy; virus

Introduction

Multipotent mesenchymal stromal cells (MSC)¹, are non-hematopoietic cells present in the human bone marrow. MSC can be readily cultured, have extensive proliferative capacity and can differentiate into more mature lineages, such as fat, cartilage and bone prompting clinical trials using MSC for tissue engineering²⁻⁵. The cells seem to have immunomodulatory properties: MSC infusions reverse therapy resistant grade IV acute graft-versus-host disease (GvHD) of skin, gut and liver^{6, 7}. The risk of transmitting viruses from *ex vivo* expanded MSC is of particular concern in hematopoietic stem cell transplant (HSCT) recipients with severe GvHD, because these individuals with GvHD-associated immunodeficiency compounding the effect of multiple immunosuppressive agents, are particularly at risk from viral infection⁸⁻¹⁰. We have found that MSC are permissive for herpes simplex and cytomegalovirus, but not Epstein-Barr virus infection. However, herpes viruses have not been found in MSC derived from healthy donors¹¹.

B19 is a non-enveloped single-stranded DNA virus with pronounced tropism for erythroid precursors and megakaryocytes which express the erythrocyte P antigen (globoside), known as the B19 receptor¹². Furthermore, the $\alpha 5 \beta 1$ integrin (fibronectin) and the Ku80 autoantigen have been described as B19 co-receptors^{13, 14}. The replication of B19 is restricted. Only a few permissive cell lines have been described, including erythroleukemia and megakaryoblastoid cell lines^{15, 16}. B19 is common worldwide and the seroprevalence increases with age: 15% of preschool children, 50% of young adults and approximately 85% of the elderly. Primary infection in children can manifest as erythema infectiosum ("Fifth Disease"), which is normally self-limiting with mild symptoms and results in life-long immunity¹⁷. Clearance of the virus is slow and largely mediated by neutralizing antibodies¹⁸. Primary infection with B19 can cause aplastic crises in individuals with high red cell turnover and chronic red cell aplasia, or even severe pancytopenia in immunocompromised individuals^{17, 19}. The virus is resistant to inactivation used in the procurement of blood and hematopoietic stem cell products, leading to a known risk of B19 transmission²⁰. There is therefore a risk that patients naïve to B19 with hypogammaglobulinemia following HSCT may develop severe cytopenias¹⁷.

Endothelial cells and fetal myocytes have been reported to host B19^{21, 22}. The virus can persist in the bone marrow and recently Rollin *et al.* detected human parvovirus B19 in MSC from 1/18 healthy donors²³⁻²⁵.

We therefore screened clinical grade MSC products used to treat patients with GvHD for presence of B19. Here we describe persistence of B19 in MSCs that could infect hematopoietic cells *in vitro*. However, we did not observe any clinical consequences of MSC infusions containing B19 to immunocompromised recipients.

Subjects, material and methods

Donors and recipients

The MSC donors (n=20) were selected randomly and considered healthy after assessment of medical history, physical examination and serological screening for HIV (n=20), hepatitis (n=20) and herpes (n=15) viruses. The recipients underwent HSCT at the Center for Allogeneic Stem Cell Transplantation, Karolinska University Hospital Huddinge, between 2002 and 2006. MSC were used as prophylaxis or treatment of GvHD. Second and/or third passage MSC were given in dosages of $\sim 1-2 \times 10^6$ /kg. Patients who had received the B19 contaminated cells were assessed retrospectively for persisting anemia, leucopenia, thrombocytopenia, rash or arthritis, possibly related to B19 infection. Donors and recipients gave informed consent and the study had approval by the Regional Ethics Review Board.

Isolation and ex vivo culture of MSC

To isolate MSC, bone marrow aspirates of 50 (range 16-80) mL were taken from the iliac crest of healthy donors, 9 males and 11 females, with a median age of 40 (range 24-66) years. The clinical grade MSC expansion was performed according to the guidelines of the MSC consortium of the European Blood and Marrow Transplantation Group, and was approved by the Swedish Medical Products Agency, as previously described in detail^{7, 26}.

To assess the growth of the MSC, time to first passage and doubling time in first and second passage were recorded. The doubling time was calculated using the following equation: $\text{doubling time} = t / (\log_2(y/x))$, t = time in culture, y = cell count at confluence, x = cell count at start. Adipogenic and osteogenic differentiation after induction was evaluated as previously described²⁷.

Characterized by flow cytometry, the MSC uniformly fulfilled MSC criteria¹. The MSC suspensions were culture-negative for bacteria and fungi, and PCR negative for *Mycoplasma pneumoniae*^{7, 26}.

PCR detection of B19 DNA and anti B19 IgG serology

For quantification of parvovirus B19 DNA, a human parvovirus genotype 1-, 2- and 3-specific TaqMan real-time PCR assay was used, as previously described¹⁸. DNA from 10^5 MSC and DNA from 200 μ L of serum samples were extracted by an automated MagnaPure extractor (Roche Diagnostics Scandinavia, Stockholm, Sweden) using the "LC Total Nucleic Acid Isolation Kit" (Roche Diagnostics).

Serum samples were analyzed for anti-B19 IgG using a commercial EIA (Biotrin International).

Isolation of peripheral blood lymphocytes and mixed lymphocyte cultures

Peripheral blood lymphocytes (PBL) were isolated from the HSCT recipients that received the B19 positive MSC and used in mixed lymphocyte cultures (MLC), to determine responsiveness to allogeneic cells, as described elsewhere^{11, 28}.

Determination of P antigen, Ku80 and glycophorin A expression

The expression of P antigen by MSC was determined by flow cytometry. MSC were incubated with plasma from an individual with anti-P antigen antibodies (optimized to 1/20; anti-PP1Pk, i.e. anti-Tja, blood group AB Rh- without anti-HLA-antibodies; Department of Transfusion Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden) and polyclonal rabbit anti-globoside antibody (optimized to 1/10; Matreya LCC, Pleasant Gap, PA, USA), respectively. Thereafter, the MSC were incubated with monoclonal fluoresceinated anti-human immunoglobulin antibodies (optimized to 200 µg/mL; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and anti-rabbit immunoglobulin (optimized to 100 µg/mL; Sigma-Aldrich, Saint Louis, MO, USA), respectively. The plasma from the individual with anti-P antigen antibodies was also used after absorption with erythrocytes expressing these antigens as a specificity control. Ku80 expression was investigated using a monoclonal mouse anti-Ku80 antibody (optimized to 20 µg/mL; Abcam plc, Cambridge, UK) and a monoclonal anti-mouse antibody (optimized to 200 µg/mL; Jackson ImmunoResearch Laboratories). Glycophorin A (CD235a) expression was determined by incubation with a monoclonal phycoerythrin-labelled anti-CD235a antibody (optimized to 2 µg/mL; BD Biosciences, San Jose, CA, USA). In all experiments, six MSC were tested and three bone marrow cells were used as control cells. Finally, labelled cells were assayed in a flow cytometer (BD Biosciences). Fluorescence signals from 5×10^4 cells were counted and the percentage of fluorescence positive cells was recorded.

Evaluation of B19 transmission

To evaluate possible transmission of B19, the B19 positive adherent MSC were co-cultured in RPMI 1640 medium supplemented with 2 mM HEPES, 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine (Gibco-BRL, Life Technologies, Paisley, UK), and 10% heat-inactivated pooled human AB-serum, with P antigen positive bone marrow cells (n=3) in suspension for 3 days. Bone marrow cells incubated with B19 rich plasma (lot BPL9; kindly provided by Dr. Kerr, Biotrin International; used 10 genomic equivalents (geq)/target cell) were used as control. Adherent B19 negative MSC (n=3) were also cocultured with the B19 positive MSC in transwell inserts (1:1) and, as control, the MSC were exposed to B19 rich plasma (10 geq/cell). In an additional assay, blocking of possible B19 transmission was investigated using anti-B19 antibodies (optimized to 4 µg/mL; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in the culture medium of B19 positive MSC/bone marrow cell co-cultures (n=3) for 3 days. Bone marrow cells were dried on microscopy slides and adherent MSC were cultured on slides. Both types of slides were fixed in methanolacetone solution at -20° C. Slides were stained with a mouse anti-B19 antibody (optimized to 2 µg/mL; Santa Cruz Biotechnology) and a secondary fluoresceinated anti-mouse immunoglobulin antibody

(optimized to 100 µg/mL; Jackson ImmunoResearch). After mounting with medium containing DAPI (Vector Laboratories, Burlingame, CA, USA) to visualize the nuclei, slides were read independently by two of the authors. For slides with bone marrow cells and MSC, more than 500 and 150 cells were counted, respectively, and percentage fluorescein positive cells recorded. The analysis was performed using a fluorescence microscope (DMRXA; Leica Microsystems, Wetzlar, Germany) at 22° C with a 40× oil objective (PL Fluotar Oil; Leica Microsystems) and filters for fluorescein and DAPI (Chroma Technology, Rockingham, VT, USA). Images were captured using a camera (S/N 370 KL 0565; The Cooke Corp, Romulus, MI, USA) and SlideBook 2.1.5 software (Intelligent Imaging Innovations, Denver, CO, USA). Finally, images were post-processed and mounted using Photoshop 3.0 (Adobe Systems, San Jose, CA, USA).

Results

Expected serological results of the donors and presence of B19 DNA in MSC from one donor

The serological investigation of anti-B19 IgG was possible in 75% (15/20) of the donors included in this study who showed a seroprevalence of 47%. DNA was successfully extracted from donor serum and MSC (data not shown). None of the donors had B19 DNA present in their serum. However, in one of the seropositive donor MSC (K11), B19 DNA was found present in 1×10^3 geq/ 10^5 cells.

No differences in growth and phenotype of the MSC

The B19 DNA positive MSC required 14 days in culture to first passage. This was within the range of 8-18 days for the other 19 MSC. Similarly, cell doubling time (mean $\pm$ SD) in first and second passage was comparable (3.1 and 4.1 days, respectively, for the B19 DNA positive MSC, compared to 3.7 ± 1.7 and 3.6 ± 0.8 days, respectively, for the B19 negative MSC). Cells from seronegative and seropositive donors exhibited a doubling time of 4.4 ± 1.9 and 3.0 ± 1.0 days, respectively, in first passage and 3.7 ± 0.7 and 3.6 ± 0.9 days, respectively, in second passage.

All expanded MSC showed the same phenotype assessed by flow cytometry of surface markers. No differences in differentiation between the B19 positive and the other MSC could be demonstrated by morphological examination and spectrophotometry. This was also valid for cells from seronegative donors compared to cells from seropositive donors (data not shown).

MSC express P antigen, Ku80 and glycophorin A

We used flow cytometry to detect P antigen on MSC using plasma from an individual with anti-P antibodies and a commercial anti-globoside antibody. P antigen was expressed by all MSC (n=6) tested, but less strongly than on bone marrow cell controls (n=3); MSC displayed low expression using the anti-P antibody plasma or the commercial anti-globoside antibody, and bone marrow cells displayed high expression. After absorption, with erythrocytes, of the anti-P antibody plasma, the MSC expression was reduced to background (secondary antibody). The B19 PCR positive MSC K11, displayed low expression using the

anti-globoside antibody. MSC expressed Ku80 in higher levels than bone marrow cells. In contrast to bone marrow cells, which displayed high expression of glycophorin A using the anti-glycophorin A antibody, MSC displayed low levels (Figure 1).

MSC can transmit B19 to bone marrow cells in vitro

Bone marrow cells from three individuals exposed to B19 rich plasma for three days were 10, 11 and 12% positive for intracellular B19 antigens by immunofluorescence compared to less than 1% for non-exposed cells (Figure 2). In parallel, when the bone marrow cells from these subjects were co-cultured with the B19 positive MSC K11: 6, 6 and 7% of the cells were antigen positive for B19 by immunofluorescence. In contrast, co-cultures to which anti-B19 antibody was added showed only background (<1%) fluorescence. B19 exposed MSC showed reduced viability (low number of cells) compared to the unexposed or antibody-treated controls.

Immunofluorescence analysis of the B19 PCR positive MSC resulted in 3% positive cells. Exposure of MSC from three donors to B19 rich plasma resulted in 41, 48 and 58% B19 positive MSC as compared to background levels (<2%) in unexposed controls, and could be blocked by anti-B19 antibody. We did not observe transmission of B19 from the B19 positive MSC to 3 B19 negative MSC in transwell co-cultures.

Clinical outcome of recipients of parvovirus contaminated MSC

MSC K11, B19 positive by PCR, was given to two individuals in a clinical trial to treat severe refractory acute GvHD. Characteristics of patients, HSCT and MSC therapy are shown in Table 1. One of the donor-recipient pairs were mismatched in B19 serology. No B19 could be detected by PCR in neither donors nor recipients pre-HSCT. Both recipients were immunocompromised by the means of leucopenia, subnormal immunoglobulin levels and poor responses in mixed lymphocyte cultures (MLC). However, none of them had any clinical signs of B19 infection, blood counts remained stable, there was no B19 seroconversion and no B19 DNA could be detected in blood post-MSC transplantation. The patients succumbed from relapse and septicemia, respectively. Both causes of death were most likely unrelated to infusion of B19 positive MSC.

Discussion

Plentz *et al.* showed that 1% of all blood products are positive for B19 DNA and the highest incidence (17.6%) was found in hematopoietic stem cells mobilized to the periphery and in bone marrow²⁰. Hence, many manufacturers of blood and plasma derivatives screen their products for B19 by quantitative PCR²⁹. B19 infection could pose a risk for severe cytopenia in immunosuppressed HSCT recipients with persistence of the virus in the bone marrow³⁰. Since B19 was reported to occur in bone marrow derived MSC²³, we retrospectively screened for B19 in 20 MSC used to treat GvHD in HSCT recipients. Anti-B19 IgG was detected in about 50% of the healthy MSC donors, which correlates with the estimated seroprevalence in the adult population¹⁷. None of the MSC donors were viremic as they tested negative for B19 DNA in serum. However, we found B19 DNA in MSC from one donor. Hematopoietic contamination as a source of B19 was unlikely since the presence

of such cells is excluded by flow cytometry before release of clinical MSC batches. Cassinotti *et al.* demonstrated that B19 can persist in the bone marrow of healthy and asymptomatic individuals²⁴. Cells expressing the P antigen are permissive for B19 entry^{12,31}. In absence of P antigen, the $\alpha 5\beta 1$ integrin and the Ku80 can serve as B19 co-receptors^{13, 14}. $\alpha 5\beta 1$ integrin expression on MSC was recently reported³². We therefore tested MSC from six donors, including the B19 positive cells, for surface expression of P antigen and Ku80. All MSC expressed the antigens/B19 receptors. To clarify whether the MSC share other features of erythroid cells, glycophorin A expression was determined but found to be expressed at low levels.

B19 positive MSC infected bone marrow cells in a manner similar to B19 rich plasma. MSC could also be infected by the B19 rich plasma but exhibited poor survival, suggesting a cytopathic effect. However, the B19 DNA positive MSC could not infect other MSC. This may be explained by low virus concentrations, since the B19 rich plasma could infect the MSC, together with weaker expression of P antigen on MSC compared to bone marrow cells. Furthermore, B19 positive and negative MSC were separated in a transwell system which prevented cell-cell contact which may be required for viral transmission. Compared to bone marrow cells, MSC exposed to B19 rich plasma showed stronger positivity for B19 antigens by immunofluorescence. This may be explained by the relatively higher proliferative and metabolic activity of *ex vivo* cultured MSC. We have shown that MSC to a certain extent are permissive. The B19 is known to have an extreme tropism for human erythroid cells and only a few cell lines support semipermissive infection *in vitro*^{15, 16}. Recently, Wong *et al* reported that *ex vivo* generated CD36+ erythroid progenitors may be used to study B19 replication³³. The unavailability of *in vitro* culture systems for productively growth of B19 has limited the research. The transmission of B19 was inhibited by anti-B19 antibodies, suggesting that endogenous B19 neutralizing antibodies or intravenous administrations of pooled immunoglobulins may be protective *in vivo*. Thus, MSC may be a natural reservoir for persistent B19. This is in analogy with the findings of Scadden *et al.*, who described bone marrow stromal cells as a reservoir of HIV, passing the virus to both myeloid and lymphoid cells³⁴. Hence, MSC and the bone marrow stroma might be a site of persistent viruses. In the absence of reliable long-term culture systems to generate B19 *in vitro*, MSC, which we show here can be readily infected by B19, could be used to propagate the virus long-term. Our studies, however, do not explain why only a small minority of seropositive individuals shows viral persistence in the marrow.

The viral load in the B19 positive MSC was 1×10^3 geq/ 10^5 cells, roughly comparable to the median viral load 0.7×10^3 geq/mL in blood components reported by Plentz *et al*²⁰. Thus infectivity of the MSC should be comparable to that of blood products. In the study by Plentz, 12% of the patients, the majority undergoing HSCT, in a hematological ward were exposed to B19 contaminated products. No patient developed clinical disease, but one developed viremia²⁰. We report two heavily immunocompromised patients with severe GvHD that were exposed to the B19 contaminated MSC. Both patients were within three months of HSCT with severe GvHD. They were leucopenic, deficient in immunoglobulins and showed functional T lymphocyte deficiency by MLR at the time of MSC infusion. Neither patient developed clinical signs of infection nor had detectable B19 DNA or anti-

B19 IgG post-MSCT infusion, indicating that the patients never became infected. It is possible that the viral transmission was below the threshold for established infection. In a case where the HSCT recipient developed B19 infection, the donor was found to have asymptomatic viremia suggesting that much higher viral loads are required³⁵. However, quantitative information relating viral load to infectivity is lacking and it is unclear how much risk immunodeficient HSCT patients are from B19 infection. The low immunoglobulin levels encountered after transplant might increase the risk for new infection, or reactivation in asymptomatic carriers. However, B19 infections do not constitute a major, or perhaps are an under-diagnosed, clinical problem in HSCT recipients. Cytopenias and viral illness due to primary or reactivated B19 infection have been described¹⁹. B19 infected MSC displayed low viability, which also may explain the absence of infection in our patients.

Our data support the possibility that B19 has low pathogenicity even in highly immunosuppressed individuals receiving MSC which may themselves further reduce immunity against viruses¹¹. Nevertheless, since data is lacking it seems prudent to screen MSC products from seropositive donors for B19 DNA and only give virus negative products, or as a minimum, infuse MSC containing B19 only to patients with a measurable titer of antibodies to B19. Further investigations of the relationship of MSC to viral persistence and screening of MSC products for contaminating viruses are warranted.

Acknowledgments

We hereby acknowledge the Swedish Cancer Society, the Swedish Research Council, the Tobias Foundation, the Cancer Society in Stockholm, the Swedish Society of Medicine, the Stockholm County Council, the Sven and Ebba-Christina Hagbergs Foundation (KLB), the Children's Cancer Foundation (KB and KLB), Signe och Olof Wallenius stiftelse, Stiftelsen Sigurd och Elsa Goljes Minne and the Claes Högman's SAGMANscholarship/Fenwal Blood Technologies Inc. (MS), and Karolinska Institutet (MS, KLB and KB), for unrestricted financial support.

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Figure 1. P antigen, Ku80 and glycophorin A expression on MSC determined by flow cytometry (a) Unfractionated bone marrow cells and (b) MSC expressed P antigen as investigated by a commercial anti-globoside antibody. However, the MSC were less positive than the bone marrow cells. (c) Expression of P antigen on MSC could also be demonstrated using patient plasma with anti-P antigen antibodies. (d) The specificity of the plasma was determined by reduction of positivity after absorption with erythrocytes. (e) Unfractionated bone marrow and (f) MSC expressed Ku80, although MSC at slightly higher levels. (g) Unfractionated bone marrow cells expressed glycophorin A, whilst (h) MSC only displayed a weak positivity. Matched isotype controls defined the quadrants. Values on Y-axis indicate intensity log. Representative dot-plots of cells from 6 different donors are shown.

Figure 2. Parvovirus B19 immunostaining to investigate transmission from MSC

(a) Unexposed bone marrow cells were negative for B19. (b) Bone marrow cells incubated with B19 rich plasma were positive, green, for B19 fluorescence. (c) Transmission of B19 was shown, since positive cells, could be detected after co-culturing B19- negative bone marrow cells with B19-positive MSC. (d) The transmission of B19 could be inhibited by addition of anti-B19 antibodies in the medium of the co-culture. (e) Unexposed MSC were negative for B19. (f) MSC incubated with B19 rich plasma were found positive, for B19 green grainy fluorescence. (g) No transmission of B19 from the B19 positive MSC to B19 negative MSC could be detected after co-culture. Representative photos are shown.

Table 1
Hematopoietic stem cell transplant patients that received the parvovirus B19 positive MSC K11 as treatment of graft-versus-host-disease

Patient		
Unique patient number	1044	1082
Sex/Age (years)	M/60	F/65
Diagnosis	Myeloma	CLL
HSCT		
HLA match	HLA-id Sib	HLA-id Sib
Conditioning	RIC: Flu+TBI	RIC: Flu+Cy
Cell source	PBSC	PBSC
GvHD prophylaxis	CsA+MMF	CsA+MTX
Day of GvHD diagnosis	51	25
Grade of acute GvHD	III	III
MSC transplantation		
Indication	GvHD	GvHD
HLA match	MM	MM
Day of first MSC infusion	77	32
Number of MSC infusions	1	3*
MSC passage	2	2+3/3/2+3
MSC dose ($\times 10^6$ /kg)	0.9	1.7/1.7/0.9
GvDH response to MSC infusion	CR	PR
B19 investigations and symptoms		
HSC donor anti-parvovirus B19 IgG serology	+	–
HSC donor serum load of parvovirus B19 DNA ($\times 10^3$ geq/mL)	–	–
Recipient anti-parvovirus B19 IgG serology, pre-tx	–	+
Recipient anti-parvovirus B19 IgG serology, 1 month post-tx	–	–
Recipient anti-parvovirus B19 IgG serology, 3 months post-tx	–	–
Recipient serum load of parvovirus B19 DNA ($\times 10^3$ geq/mL), pre-tx	–	–
Recipient serum load of parvovirus B19 DNA ($\times 10^3$ geq/mL), 1 month post-tx	–	–
Recipient serum load of parvovirus B19 DNA ($\times 10^3$ geq/mL), 3 months post-tx	–	–
Clinical symptoms of parvovirus B19 disease	None	None
Recipient immunocompetence at MSC transplantation		
Lymphocyte proliferation in allo-MLC (% of positive control)	22%	14%
IgG level (g/L, reference interval: 6.1–14.9)	3.4	5.1
IgA level (g/L, reference interval: 0.88–4.5)	0.41	0.91
IgM level (g/L, reference interval: 0.27–2.10)	0.49	0.34
White blood cell count ($\times 10^9$ /L, reference interval 3.5–8.8)	8.7	1.8
Absolute neutrophil count ($\times 10^9$ /L, reference interval 1.6–7.5)	7.6	0.8
Blood parameters[†]		
Hemoglobin (g/L, reference interval: F 117–153, M 134–170)	95/90/85	114/117/124

White blood cell count ($\times 10^9/L$, reference interval: 3.5–8.8)	8.7/5.4/5.0	1.8/9.1/9.9
Platelet count ($\times 10^9/L$, reference interval: F 165–387, M 145–348)	104/119/78	55/96/92
Outcome	$\dagger$, relapse, day 773	$\dagger$, septicemia, fungi, day 120

M, male; F, female; CLL, chronic lymphatic leukemia; HLA-id Sib, HLA-identical sibling; RIC, reduced intensity conditioning; Flu, fludarabine; TBI, total body irradiation; Cy, cyclophosphamide; PBSC, hematopoietic stem cells mobilized to peripheral blood; GvHD, graft-versus-host-disease; CsA, cyclosporine A; MMF, mycophenolate mofetil; MTX, methotrexate; MM, HLA mismatched unrelated; CR, complete response; PR, partial response; HSC, hematopoietic stem cell; geq, genome equivalents; pre-tx, pre-transplantation; post-tx, posttransplantation; allo-MLC, allogeneic mixed lymphocyte culture;

$\dagger$ deceased.

* Patient 1082 received three infusions of MSC. The first infusion was MSC K11, which later was found to be contaminated with parvovirus B19.

$\square$ measured at MSC infusion, and approximately 1 and 2 weeks post-MS*C* infusion.