Impact of allogeneic hematopoietic stem cell transplantation on the biological parameters of immunity in patients with chronic granulomatous disease

Reconstituția imunologică completă – factor cheie pentru evoluția pacienților cu boală granulomatoasă cronică după transplant de celule stem hematopoietice

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Abstract

Donor derived neutrophil reconstitution, defined as the normalization of the neutrophil counts and function after allogeneic hematopoietic stem cell transplantation (HSCT), represents a prerequisite for the cure of patients with chronic granulomatous disease (CGD), expressed by a normalization of the nitroblue tetrazolium assay (NBT test), of the dihydro-rhodamine 123 (DHR) oxidation and of other confirmatory tests. These investigations together with the flowcytometric analysis of the lymphocyte subsets reconstitution at repeated post-transplant intervals predict the outcome after HSCT in these patients. Because a normalization of phagocyte activity implies a complete neutrophil engraftment with the presence of a stable and functional donor-derived neutrophil population in the transplant recipient, lineage-specific chimerism analysis represents also an important follow-up tool. All the above mentioned parameters have been investigated in two patients with CGD with a long history of life-threatening fungal and bacterial infections non-responsive to specific anti-infectious therapies, in whom the HSCT represented the only chance for cure. We showed that the hematological reconstitution in terms of absolute neutrophil count and function was very fast, within 1 month post-transplant, leading to a decisive and immediate clinical improvement of the patients, despite the delayed reconstitution of the lymphocyte subsets. The normalization of the neutrophil function tests in both patients correlated well with the achieved full donor chimerism and with the clinical outcome of these patients after the transplant. Further controlled clinical studies are warranted in order to comparatively assess the reconstitution of the lymphocyte subsets in bone marrow versus peripheral blood stem cell transplantation for CGD.

Keywords: Analysis of immune reconstitution, hematopoietic chimerism analysis, leukocyte oxidative burst chronic granulomatous disease, hematopoietic stem cell transplantation.

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Rezumat

Reconstituția imunologică completă după transplant alogenic de celule stem hematopoietice (TCSH), definită prin normalizarea subpopulațiilor limfocitare dar totodată și imunității nespecifice (granulocite și monocite), reprezintă o premisă pentru o reactivitate imunologică adecvată. În cazul bolii granulomatouse cronice (BGC) aceasta este exprimată prin normalizarea testului nitroblue tetrazolium (NBT), a exploziei oxidative a neutrofilelor precum a testului de chemiluminescență. Efectuarea acestor investigații la anumite perioade post-transplant are valoare prognostică pentru evoluția pacienților cu BGC. Deoarece normalizarea activității fagocitare după transplant implică o greață completă a neutrofilelor cu prezența unei granulopoioze stabilize derive de la donor, chimerismul specific de linie reprezintă de asemenea o analiză importantă pentru monitorizare. Toți parametrii menționați anterior au fost investigați la doi pacienți cu BGC și cu un istoric lung de infecții fungice și bacteriene, non-responsive la terapii specifice și cu care TCSH a reprezentat singura ansă pentru vindecare. Normalizarea funcției neutrofilelor la ambiți pacienților s-a corelat cu chimerismul complet de donor precum și cu evoluția clinică favorabilă a acestora după transplant.

Cuvinte cheie: analiza reconstituției imunologice, analiza chimerismului hematopoietic, explozie oxidativă a neutrofilelor, boala granulomatoasa cronica, transplant de celule stem hematopoietice

Introduction

Chronic granulomatous disease (CGD) is a genetically heterogeneous condition characterized by recurrent life-threatening bacterial and fungal infections and formation of granuloma. The disease develops consequently to defects in the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, resulting in an inability to produce the superoxide anion necessary for normal destruction of bacterial and fungal microorganisms. In addition, these defects predispose to granulomatous complications and autoimmune diseases (1). Mutations in at least 5 different genes involved in the assembly and activation of the NADPH oxidase can lead to CGD. The gene encoding the enzymatic center of the NADPH oxidase, gp91phox, is located on the X chromosome and accounts for the majority of the cases. Autosomal forms occur from mutations in p47phox p67phox, p22phox, or p40phox, the latter being only recently described. In general, gp91phox-deficient patients are the most severely affected, whereas patients with mutations in p47phox seem to have the best overall outcomes. Deficiency in p40phox might predispose to more gastrointestinal disease and fewer infections (2). Diagnostic tests for CGD rely on various measures of superoxide production. These include direct measurement of superoxide production, cytochrome c reduction assay, chemiluminescence, nitroblue tetrazolium (NBT) reduction test, and dihydrorhodamine 123 (DHR) oxidation test. The quantitative dihydrorhodamine 123 flow cytometry assay is today’s most accurate diagnostic test for CGD (3). These neutrophil function assays are always to be completed by confirmatory tests such as immunoblot. Still there is a limitation of this technique because it cannot distinguish between the X-linked gp91phox defect and the p22phox autosomal recessive defect (4). Sequencing of the patient’s phox genes to determine the exact genetic defect is most reliable but not necessary for current clinical practice (5).

Reduction in mortality and morbidity in patients with CGD is nowadays mainly attributable to improved antimicrobial prophylaxis and aggressive treatment of infections (6,7). Nevertheless, allogeneic hematopoietic stem cell transplantation (alloHSCT) remains the only way of cure (1,3,5,8). Hematologic reconstitution after alloHSCT leads to normalization of the neutrophil function along with the cure of the pretransplant infectious complications in the case of
a stable donor cell engraftment. In the situation of engraftment and full donor chimerism, the full normalization of the neutrophil function is prompt in contrast with the delayed reconstitution of lymphocyte subsets. The correlation between the dynamics of the immune reconstitution and the clinical outcome of some patients with pre-transplant severe infectious complications represented the objective of our study.

**Patients and methods**

We present the schedule of laboratory investigations and the outcome of 2 patients with x-linked CGD, who received an allogeneic HSCT in the Department of Bone Marrow Transplantation of the III Clinic of Pediatrics Timisoara, using G-CSF primed peripheral blood stem cells from a sibling brother in one case and bone marrow in the other one. The aims of this study were the assessment of the importance of hematopoietic chimerism analysis in connection with the analysis of the immune reconstitution (including neutrophil function tests) in predicting the outcome of these patients. The characteristics of the patients are shown in Table 1. Post-transplant chimerism analysis, flowcytometric assessment of the immune reconstitution as well as flowcytometric 123 (DHR) tests were performed after the transplantation and their results correlated with the clinical outcome.

**Patients**

*Patient 1* is a 13 year-old boy presenting since the age of 3 weeks repeated episodes of fever, often of unknown origin, abscesses, phlegmons, pneumonia and osteomyelitis, requiring almost

### Table 1. Characteristics of the patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Medical history and treatment</th>
<th>Specific diagnostic investigations</th>
<th>CMV serology</th>
<th>EBV serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>13 years</td>
<td>- repeated episodes of fever since the age of 3 weeks; cervical adenophlegmons at 16 months of age, hepatosplenomegaly; several cutaneous abscedations at the age of 6; recurrent respiratory infections, weight loss since the age of 8 years; pneumonia of the left lower lobe with abscess formation and encapsulated pleural effusion; bronchoalveolar lavage (BAL) was positive for <em>Klebsiella pneumoniae</em> and <em>Candida albicans</em>; at the age of 9 - dorsal spine (D5-D7) osteomyelitis, pulmonary granulomata as well as extensive para-vertebral and posterior paracostal extrapleural abscesses; BAL revealed abundance of <em>Aspergillus fumigatus</em></td>
<td>- Nitroblue tetrazolium (NBT) test - pathologic; - chemiluminescence absent; - 123 - dihydro-rhodamine (123 DHR) test absent oxidant production. - the mutation of the gene CYBB encoding the gp91phox on 4th exon, c.271C&gt;T, p.R91X</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Patient 2</td>
<td>7 years</td>
<td>- repeated episodes of bloody diarrhea, pneumonia, otitis media from 2 weeks of age; several pulmonary, urinary tract, central venous line infections with <em>E. Coli, Klebsiella spp., Staphylococcus aureus, Cryptococcus neoformans</em> starting with the age of 2 years; at 6 years of age pulmonary biopsy performed from a suspect lesion revealed <em>Aspergillus fumigatus and Serratia marcescens</em></td>
<td>- NBT test - pathologic; - direct superoxide release test (0.42nmol/2 million granulocytes/15min); - the phagocyte oxidase mutation gp91phox was also found on genetic analysis</td>
<td>(+)</td>
<td>(+)</td>
</tr>
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</table>
continuous antibiotics therapy. The medical history is detailed in Table 2. At the age of 9 years extensive investigations including CT-scans, MRI, bone scintigraphy and a PET-CT scan were initiated in order to diagnose the septic determinations. It was then, when the cytological analysis of biological material from a thoracic collection and from bronchoalveolar lavage (BAL) revealed the presence of *Aspergillus fumigatus*. These findings prompted further specific investigations: the NBT test, 123-DHR-test, and finally the molecular genetic investigation revealing the mutation of the gene CYBB encoding the gp91phox (Table 1). Long-term (23 months) therapy of fungal infections with voriconazole, caspofungine, posaconazole could not control the infectious complications. HLA high-resolution molecular typing performed in our centre revealed 10/10 compatibility with the patient’s brother (for HLA-A, B, C, DRB1, DQB1 alleles). Long-term antifungal therapy for as long as 5 years with voriconazole, caspofungine and posaconazole, alternatively or in combination, justified the indication of transplantation of bone marrow hematopoietic stem cells from the sibling (Table 2).

The conditioning regimen, CD34+ and CD3+cell content of the grafts as well as the immunosuppressive prophylaxis of graft versus host disease are presented in Table 2.

### Methods

**HLA DNA typing**

Genotyping of HLA alleles was performed with molecular biology methods, namely the high resolution SSP-PCR (sequence-specific primer PCR) amplification technique for HLA A, B, C, DRB1, DQB1. Isolation of DNA was automated using the MagNaPure device, obtaining a DNA concentration of 53.6 ng/µL and a 260/280 fraction of 1.86. Invitrogen reagents were used for PCR.

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**Table 2. Conditioning regimen, stem cell and T-cell doses, immunosuppressive therapy**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Conditioning</th>
<th>Imunosuppression</th>
<th>Graft type and content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug/dose (mg/kg/day)</td>
<td>Days</td>
<td>CD34+ (no. of cells/kg)</td>
</tr>
<tr>
<td>1</td>
<td>i.v.Bu/3.2</td>
<td>-9, -8, -7, -6</td>
<td>i.v./oral CsA,</td>
</tr>
<tr>
<td></td>
<td>i.v.Cy/50</td>
<td>-5, -4, -3, -2</td>
<td>oral MTX</td>
</tr>
<tr>
<td></td>
<td>MabCampath/0.1</td>
<td>-4, -3, -2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>p.o.Bu/4</td>
<td>-9, -8, -7, -6</td>
<td>i.v./oral CsA,</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Bu=busulphan, Cy=cyclophosphamide, CsA=cyclosporine A, MTX=oral methotrexate, PBSC = peripheral blood stem cells.
amplification and a GeneAmp 9700 thermocycler. AllSet Gold SSP Invitrogen, HLA High Resolution was used as reaction kit with a HLA SSP 96 well thermal tray. For the detection of the amplified specific product, electrophoresis in 2% agarose gel was performed (2 g agarose in 100 ml TBE 0.5X). Results were interpreted using the Unimatch Program.

Cell count of the graft

Regarding the method for CD34+ and CD3+ cell count in the graft for each donor, two tubes were prepared and processed: (1) double-staining CD34PE/CD45PerCP and (2) triple-staining CD4FITC/CD8PE/CD3PerCP. Samples were incubated with antibodies 20 min in the dark, at room temperature. The next step was the red cells lysis with BD Facs Lysing solution followed by centrifugation 5 min at 300g, then cell washing with cell-wash, resuspension and acquisition of 100,000 events. Cells were acquired on a four-color FACS Calibur flow cytometer (Becton Dickinson Biosciences) equipped with a 488 nm laser and analyzed with CellQuest 3.1 software.

Gating strategy applied for CD34+ cells detection was the two-platform ISHAGE protocol (CD34+ cells are detected based on four parameters: CD45PerCP/CD34PE/side and forward single light scatter). T-lymphocytes were determined as the percentage of cells that reacts with anti-CD3 FITC (fluorescein isothiocyanate).

Post-transplant chimerism analysis

Microsatellite analysis by PCR for hematopoietic chimerism was performed at CCR1/LabDia, Vienna, Austria, as described previously (9,10,11). Chimerism was prospectively analyzed on days: +30, +90, +180, +270, +365 post-transplant and yearly after, according to international guidelines (12).

Analysis of the post-transplant lymphocyte subsets analysis

Prospective immunologic reconstitution analysis of the following lymphocyte subsets was performed: CD19+, CD3+, CD3+/CD4+, CD3+/CD8+, CD3-CD16+/CD56+, CD45+RA, CD45+RO. Timing of flow cytometric investigations: days +30, +100, +180, +270, +365, +547, +760 post-HSCT and yearly after. In order to evaluate the immune reconstitution, absolute values were compared to the mean normal absolute value of the respective age (p50) (13). Peripheral blood samples were processed using a lysis/wash technique. Briefly, whole blood was incubated with antibodies at room temperature in the dark for 15 min followed by a lysing step with BD-FACS Lysing Solution (Becton Dickinson) for 15 min in the dark and a washing step using BD-CellWash (Becton Dickinson). Cells were resuspended in BDCellWash and acquired on a BD-FACSCalibur flow cytometer.

Antibody panels utilized: CD3FITC/CD16+56PE/CD45PerCP/CD19APC; CD45RAFITC/CD45ROPE/CD3PerCP/CD4APC; CD45RAFITC/CD45ROPE/CD3PerCP/CD8APC. T, B and NK cells were analyzed and expressed as percentages from lymphocytes and then calculated in absolute number from WBC value (WBC was measured on a hemocytometer). Data analysis protocol included also naive and memory T lymphocytes.

Quantitative assessment of leukocyte oxidative burst

Heparinized peripheral blood samples were analyzed using the PHAGOBURST kit (Becton Dickinson, cat. no. 341058). This test kit allows the quantitative determination of the leukocyte oxidative burst. It contains unlabelled opsonized bacteria (E. coli) as particulate stimulus, the protein kinase C ligand phorbol 12-myristate 13-acetate (PMA) as high stimulus and the chemotactic peptide N-formyl-MetLeuPhe (fMLP) as low physiological stimulus. Whole blood was incubated with various stimuli at 37°C; a sample without stimulus served as negative background control. Upon stimulation, granulocytes and monocytes produce reactive oxygen metabolites. This process is monitored by the addition and oxidation of DHR 123. The percentages of cells having produced reactive oxygen radicals were analyzed as well as their
Results

For patient 1, engraftment of neutrophils (defined as absolute neutrophil counts > 500/µL for 3 consecutive days) occurred on day +13 whereas for platelets (PLT) defined as PLT count > 20,000/µL on day +17. Lineage-specific chimerism analysis for patient 1 is presented in Figure 3. At 1 month after the transplant, granulocytes in the recipient were 96% of donor origin as well as monocytes, CD8+ lymphocytes and NK cells. CD4+ lymphocytes were 85% of donor origin. All leukocyte lineages progressed towards a full donor chimerism. At day +25 he presented acute graft-versus-host disease of the skin, grade II, which responded well to short course metilprednisolone. Superoxide anion (O2-) release, measured by a validated method of the cytochrome c reduction assay at 2 months after the transplant, revealed 41.38 nmol/2 million granulocytes/15 minutes superoxide release, a good result, which correlated well with the chimerism analysis. Post-transplant therapy consisted of immunosuppression with CsA (cyclosporine A) which was tapered after 6 months and short course methotrexate. Antifungal therapy throughout the early post-transplant period consisted of high-dose Amphotericin B and was continued up to 18 months post-transplant with posaconazole and periodical combination therapy of voriconazole and caspofungine. The clinical outcome was consistent with the recovery of granulocyte function. X-ray performed at 9 months post-transplant showed a almost complete resolution of the pulmonary findings. Also, flow cytometry assess-
ment of leukocyte oxidative burst performed at 9 months post-transplant revealed 84% oxidative cells at stimulation with \textit{E. coli} and 83% oxidative cells at stimulation with PMA. A repeated assay at 15 months post-transplant showed a clear improvement, with 93% of phagocytizing granulocytes at stimulation with \textit{E. coli} (Figures 1, 2).

Bronchoalveolar lavage performed at the same time revealed the absence of Aspergillus hyphae. Follow-up, this time non-lineage-specific, from whole blood at 3 years after the transplant revealed 97% donor cells and 3% recipient cells in peripheral blood. These findings were consistent with the complete resolution of granulomatous and of fungal pulmonary findings on the thorax CT scan, except for fibrous sequelae, bronchiectasis and pachypleuritis. Regarding the immune reconstitution of lymphocyte subsets, there was a gradual increase of CD19+ B lymphocytes which remained below the cut-off 10\textsuperscript{th} percentile value for age for over 1 year post-transplant (Figure 5). The CD4+ cell compartment normalized also towards 2 years post-transplant (Figure 5). CD8+ T cells reached the inferior normal limit at the 10\textsuperscript{th} percentile value for age (p10) at around 1 year post-transplant and gradually increased thereafter towards the p50 value for age, whereas the NK cells recovered up to the p10 value around 270 days post-HSCT, decreased at 1 year and then presented a plateau at the 25\textsuperscript{th} percentile value for age for another year and decreased at the p10 at the 3 years post-transplant analysis (Figure 5). At present, 3 years after his transplantation, the patient is in a very good clinical condition, goes to school and enjoys his childhood.

\textit{Patient 2} presented neutrophil engraftment on day +15 and platelet engraftment on day +17. Chimerism analysis for this patient showed 98% donor cells (full donor chimerism) at day +90 post-HSCT.

Post-transplant therapy consisted of immunosuppression with CsA (cyclosporine A), which began to be tapered after 3 months, and short course methotrexate. Antifungal therapy was performed throughout the early post-transplant period with a combination of voriconazole and caspofungine and continued after engraftment with voriconazole alone and after 3 months with posaconazole. A thorax CT scan performed at 3 months after the transplant revealed a marked improvement with resolution of the majority of the pulmonary lesions. Flow cytometry assessment of leukocyte oxidative burst performed for patient 2 at 1 month post-transplant revealed 97% oxidative cells at stimulation with \textit{E. coli} and 97% oxidative cells at stimulation with PMA (Figure 4).

The reconstitution analysis of the B-lymphocyte subset showed almost absent B cells at 1 month post-transplant but showing much improvement on day +100. Both, the CD3+CD4+ and the CD3+CD8+compartment presented very low absolute counts even at 3 months after the HSCT. NK cells (CD3-CD16+CD56+) were within normal limits already at 1 months post-HSCT (Figure 6). The patient is now at 4 months post-HSCT, having a very good clinical status but continuing acyclovir prophylaxis due to very low CD4+ counts.

\textbf{Discussion}

Although a rare disease, several reports in literature have shown especially in the last years that CGD can be cured by alloHSCT from sibling donors (14,15,16) and even from HLA matched unrelated donors (17). Cure by HSCT is associated in the majority of cases

![Figure 3. Patient 1 - lineage specific chimerism (STR-PCR)](image-url)
with an improved quality of life as compared to patients without a transplant receiving ongoing prophylaxis with a lifelong risk of infections and nutritional problems (18,19). Even though successful alloHSCT is followed by a rapid hematopoietic reconstitution, recovery of the immune system has been shown to depend on several factors including post-transplant hematopoietic chimerism and immunosuppression (20). Both of our patients presented >90% donor chimerism at day+90 post-transplant in all cell lineages, with neutrophils as the first cell compartment to achieve full donor chimerism, and this pattern correlated well with the fast engraftment of neutrophils (both patients received myeloablative conditioning regimens), with the normalization of the oxidative burst and improvement of their clinical status. Similarly to data reported in literature, a prompt clinical improvement was obtained with clear evidence of imagistic improvement (thorax CT-scans performed starting 1 month after the transplant showing regression of pulmonary aspergillosis), even despite the complete reconstitution of the lymphocyte subsets (1,14,19).

Patient 2 presented a normal oxidative burst already at day+30 and a good clinical improvement. Chimerism analysis performed on day +90 revealed 98% donor chimerism confirming the predictive value of this analysis when correlated with the other investigations. Since CGD is an inherited phagocyte disorder, not affecting the B- and T-cell compartment, lineage-specific chimerism for the neutrophil cell compartment could be informative enough, confirming the donor origin of phagocytizing neutrophils; still, complete serial assessment of all cell-lineages is of utmost importance for the prediction of the transplant outcome as shown in literature (10,11,21). In the majority of the cases transplanted worldwide, bone marrow was used instead of peripheral blood stem cell grafts in order to reduce the risk of GvHD. However it has been shown that earlier engraftment is achieved with PBSC grafts (22). We used two types of grafts respectively but in our cases no differences in time to neutrophil and platelet engraftment were found. However there were great differences in immune recovery of lymphocyte subsets as shown in Figures 5 and 6. There are host-specific and regimen-specific factors impacting on the reconstitution of the lymphocyte subsets: intensity of the conditioning regimen, stem cell source, recipient age, dose of mature cells contained in the graft and the presence or absence of GvHD. Because the standard procedure used worldwide by now suggests the use of myeloablative conditioning regimens, the immune regeneration relies predominantly on the homeostatic peripheral expansion and in a less extent on the thymus-dependent immune reconstitution due to drug-induced damage to the thymus, affecting CD4+ and CD8+ lymphocytes. This pattern is associ-
ated with inverted CD4/CD8 ratios and impaired immune function for as long as over 2 years after HSCT (23). Interestingly though, whereas the CD3+ T cells show a very slow recovery in the patient receiving bone marrow compared to the patient receiving PBSC, especially for the T helper cells, his CD19+ B cells and NK cells had a superior representation at day +30 and day +90 as compared to the patient transplanted with PBSC, consistent with the findings of some investigators (25). In contrast, other data supports evidence that the NK compartment presents similar reconstitution patterns in both types of graft sources (22). It has been shown that rapid reconstitution of NK cells provides clear evidence of the vitality of lymphoid stem cells in the setting of alloHSCT after myeloablative conditioning regimens (23,24). In our patient transplanted with bone marrow, the normal values of the NK cells at day +30 could be considered as predictable for a favorable outcome of the transplant but they did not enhance the reconstitution of the reconstitution of the lymphoid compartment. The lack of viral complications could probably be also attributable to the post-transplant acyclovir prophylaxis which was performed in both patients until the CD4+ count reached a stable value of 400/µL, explaining the need for analysis by flow cytometry at regular intervals after the transplant.

Our data suggests that there is a fast recovery of the neutrophil number and function after the alloHSCT leading to a prompt improvement of the clinical status of the patients despite the delayed lymphocyte reconstitution. Comparative data in literature regarding the lymphocyte reconstitution after transplantation using the two stem cell sources for patients with CGD is scarce, representing an interesting subject for further controlled studies.

Thus, chimerism analysis after alloHSCT, as well as flow cytometry analysis of the immune reconstitution, are diagnostic tools of utmost importance in the follow-up of patients with CGD undergoing alloHSCT.

We also strongly believe that alloHSCT could be a desirable treatment strategy for attempting the cure in young patients with CGD even before life-threatening invasive fungal infections and chronic inflammation induce end-organ damage, increasing the risk of transplant-related morbidity and mortality. Therefore patients with suspi-
cion of CDG should be diagnosed and transplanted as early as possible in the course of the disease in the case that they have a matched sibling donor.

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**Abbreviations**

alloHSCT – allogeneic hematopoietic stem cell transplantation;
BAL – bronchoalveolar lavage;
Bu = busulphan,
CGD – chronic granulomatous disease;
CsA – cyclosporine A;
CsA = cyclosporine A,
CT- computed tomography,
Cy = cyclophosphamide,
DHR – dihydrorhodamine;
fMLP - N-formyl-MetLeuPhe;
G-CSF - granulocyte colony stimulating factor;
GRA - granulocytes;
GvHD – graft-versus-host disease FACS – fluorescence activated cell sorting;
HLA – human leukocyte antigen;
MONO - monocytes;
MRI – magnetic resonance imaging;
NADPH - nicotinamide adenine dinucleotide phosphate;
NBT - nitroblue tetrazolium;
NK – natural killer cells
PET-CT positron emission tomography;
PMA - phorbol 12-myristate 13-acetate;

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