Quest Journals
Journal of Medical and Dental Science Research
Volume 9~ Issue 2 (2022) pp: 20-27
ISSN(Online) : 2394-076X ISSN (Print):2394-0751
www.questjournals.org

Research Paper

Recovery of $CD16^+$ $CD56^+$ Natural Killer Cells after Allogeneic Hematopoietic Stem cell Transplantation

Dr. Eida Elmansorry
Assistant Professor, Department of medical laboratories, faculty of medical technology, Tripoli University, Libya
Corresponding Author: Eida Elmansorry

Background: *Following hematopoietic stem cell transplantation (HSCT), natural killer (NK) cells recovery is the critical defence line against viral infections.* ***Methods:*** *Our study was performed between March 2008 and March 2009. The aim of this study was to evaluate the kinetics of NK regeneration post allogeneic HSCT and to correlate their recovery with factors that may affect the transplantation outcome and can increase the chance of its success. Twenty consecutive patients undergoing HSCT were included in our study. Reconstitution of $CD3^-$ $CD16^+CD56^+$ NK cells was quantified at different time points after transplantation (from day 30 today 360) using flow cytometry in peripheral blood samples. Descriptive statistics in terms of minimal, maximal, and mean values were used to describe the data.* ***Results****: Recovery of natural killer cells starts early and significant elevation in their count at three, six months was determined. After day 270, their counts reach stable values.* ***Conclusion****: NK cell recovery is transient and count of NK cells varies in the first year according to some factors such as graft versus host disease (GVHD) and type of transplantation.*
Keywords: *Allogeneic Hematopoietic Stem cell transplantation, Natural killer cells, Regeneration, graft versus host disease, Recovery, reconstitution*

R*eceived 24 Jan, 2022;* ***R****evised 04 Feb, 2022;* ***A****ccepted 06 Feb, 2022 © The author(s) 2022.*
P*ublished with open access at* ***www.questjournals.org***

I. INTRODUCTION

Immune reconstitution after allogeneic Hematopoietic stem cell transplantation (HSCT) generates from the donor-derived progenitors and the proliferation of the immune cells transferred with the graft. There is a significant difference in the kinetics of innate and adaptive immune recovery and the rapid reconstitution of monocytes and natural killer (NK) cells comparing with the delayed maturation of T and B lymphocytes, which may not complete until the first year after transplantation. Reconstitution of functional immune responses affected by many factors, particularly source of graft, graft versus host disease and/or its preventive therapy. Complete and functional recovery of both innate and adaptive immunity is necessary to limit the susceptibility to infection and to prevent relapse risk after allogeneic HSCT. Natural killer cells (NK) are innate lymphocytes capable of strong cellular killing and they can produce cytokines without prior antigen encounter [1]. Natural killer (NK) cells are generated from a lymphoid progenitor in the bone marrow [2,3].

The morphology of NK cells is similar to that of T and B lymphocytes, but they are granulated and are identified by the absence of CD3 (a surface molecule that is exclusive to T lymphocytes) and CD16 and CD56 expression (About 90% of NK cells in peripheral blood express both molecules), although there are subpopulations with the $CD16^-$ CD 56^+ and $CD16^+$ $CD56^-$ phenotypes, which exist between 5 and 10% of the total count of NK cells [4]. NK cells are the first defence line against invading pathogens (mainly viruses) and tumour cells. Their activation and inactivation depends on the recognition of human leukocyte antigen alleles through immunoglobulin-like receptors that inhibit killer cells [5]. Natural killer cells are large granular lymphocytes. They were defined as lymphocytes isolated from blood and they can destroy tumour cells without prior sensitization [6].

Reconstitution of natural killer cells is started one to two months after allogeneic HSCT. Mainly, NK cells which generated during this period do not generate from the expansion of mature NK cells in the graft, but from the maturation of progenitor cells [7,8].

Regeneration of innate immune responses is important in immunosuppressed patients and in allogeneic HSCT recipients because of their role in infection control.

Natural killer cell recovery after HSCT needs time to provide complete homeostasis of this cell line in the first years after allogeneic transplantation. Evaluation of this procedure is achieved by assessment of the transplant recipient, through laboratory analysis and clinical follow-up to improve the transplantation outcome [9].

The counts of natural killer cells can be detected by flow cytometry and lymphocyte count [10]. Natural killer cells are differentiated from T- and B- cells in that they lack expression of the T cell receptor and surface immunoglobulin. The best surface marker to identify all NK cells is CD56, or NCAM (natural cells adhesion molecule), the levels of CD56 expression differ on NK subsets. Natural killer cells are phenotypically defined as lymphocytes that are CD56$^+$ and CD3$^-$. Other surface antigens present on NK cells include CD16 (FCγIII), CD2, CD7, and CD8 [11]. After allogeneic bone marrow transplantation, NK cells are the first lymphocytes to regenerate in the peripheral blood [12].

A new study determined the importance of the immunophenotyping of natural killer cells in allogeneic bone marrow transplantation because the ability of incompletely MHC matched NK cells to reject residual leukaemia. The role of graft NK cell versus leukaemia effect was determined by in vitro proof that donor NK cell clones were able to destroy recipient leukemic blast targets [13.] The aim of this study is to analyze the kinetics of natural killer cells recovery at different time points after HSCT and to correlate their reconstitution with different factors that influence the transplantation outcome.

II. MATERIALS AND METHODS

2.1. Patients and setting:

An observational and descriptive study of patients undergoing allogeneic HSCT between March 2008 and March 20009 was carried out in laboratory of cellular therapy, Campus Virchow Clinic, Charite' University, Berlin, Germany.

An ethical committee approval was taken from institutional ethical committee. Twenty consecutive patients were included in this study. The diagnosis they were referred to the Hematopoietic Stem Cell Transplantation Unit for the following: acute lymphoblastic leukaemia, 35% (n=7); Myelodysplastic Syndrome ,15% (n=3); Acute myeloid Leukaemia ,10% (n=2); Wiscott-Aldrich syndrome, 15% (n=3); Fanconi Anemia ,10% (n=2); Chronic myeloid Leukaemia .5% (n=1); severe combined immune deficiency ,5% (n=1); X-chromosomal Adrenoleukodystrophy , 5%(n=1).Twelve patients (60%) were males and eight (40%) were females; age ranged from six months to 26 years.

Graft source was 75% (n=15) bone marrow and 25% (n=5) Peripheral Blood stem cell (PBSC). Some patients received a myeloablative regimen, while others received a reduced-intensity regimen (depending on the underlying disease) before allogeneic hematopoietic stem cell transplantation. 86% of the patients (n=18) had acute graft versus host disease, range from grade I –II, and 75% (n= 15) had grade II of chronic graft versus host disease.

The viruses' reactivity during the study period was as follow: Three patients (15%) had no virus reactivity, nine patients (45%) had one virus infection, seven patients (35%) had two virus infections, and one patient (5%) had three virus infections.

2.2 ***Sample techniques:***

Whole blood specimens were collected once from day 30 to day 360 post transplantation. Informed consent was obtained from all patients or their parents.

2.3 ***Cell Preparation:***

Patient's peripheral venous blood was collected into 10-ml Li-heparin/EDTA vacationer Becton Dickinson (BD, USA) after informed consent.

2.4 ***Antibodies used:***

The following monoclonal antibodies were obtained from Becton Dickinson (BD, USA): Fluorescein-isothiocyanate (FITC)-conjugated anti –CD3 (clone SK7), Phycoerythrin (PE)-conjugated anti-CD56 (clone MY31) and Phycoerythrin (PE) -conjugated anti-CD16 (clone 3G8)

2.5 ***Assay Principle:***

Aliquots of 50 microliter of EDTA/ Heparin blood were placed in FACS tubes (BD, USA) and stained with the appropriate antibodies (titrated for optimal concentration) ,then incubated shortly in the dark place. Finally the erythrocytes were lysed, washed, and FACS-lysing solution, (BD Pharmingen, USA) was added for the final fixation.

2.6 ***Flow Cytometry analysis:***

Patient cells were stained with CD3-FITC, CD16 –PE, and CD56 –PE antibodies as previously described. Cells were analyzed on FACS CAN (BD, USA) flow cytometer .Data was further analyzed using Cell Quest program software. NK cells populations (CD3$^-$CD16$^+$ CD56$^+$) for every patient were gated and quantified every month from day 30 until day 360 post transplant and absolute counts per microliter or as percentage of whole blood were determined.

3. Statistical analysis:

Statistics (means, minimal, and maximal values) were used to describe patient baseline characteristics. Results are presented as mean values of NK percentages, and p-values.

Data was analyzed using SPSS version 18. Student's *t*-test was used to ascertain the significance of differences between mean values of two continuous variables and confirmed by nonparametric Mann-Whitney test.

III. Result

Table 1: Shows Patients and transplant characteristics

	Number (%)
Males **Females**	12(60%) 8(40%)
Age (mean, min-max)	10.8(0.5-26)
Donor age (mean, min-max)	30(7-50)
Stem cell source Peripheral blood Bone marrow	 5(25%) 15(75%)
Acute GVHD (grade I-II) **Chronic GVHD**	18(86%) 15(75%)
Hematological disease Acute lymphoblastic leukaemia Myelodysplastic Syndrome Acute myeloid Leukaemia Wiscott-Aldrich syndrome Fanconi Anemia Chronic myeloid Leukaemia severe combined immune deficiency X-chromosomal Adrenoleukodystrophy	 7(35%) 3(15%) 2(10%) 3(15%) 2(10%) 1(5%) 1(5%) 1(5%)

Reconstitution of Natural killer cells (NK):

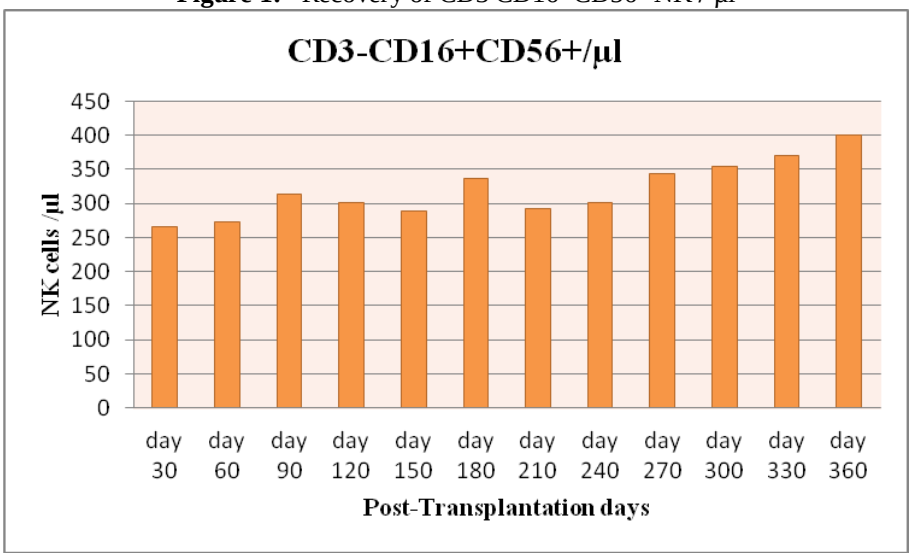
To analyze NK reconstitution kinetics in all 20 patients, frequencies of NK cells lacking CD3 expressing CD16$^+$ and CD56$^+$ surface markers were measured in whole blood from day 30, day 60, day 90, day 120, day 150, day 180, day 210, day 240, day 270, day 300, day 330, and day 360 post HSCT . Percentages of CD16$^+$ CD56$^+$ cells were measured and presented as mean, minimal, and maximal values.

The recovery of CD3$^-$CD16$^+$ CD56$^+$ NK / µl was measured at different time points after transplantation. There was a significant elevating in their levels at day 90 ($P \leq 0.002$), day 180($P \leq 0.001$), and from day 270 to day 360($P \leq 0.009$).

Table 2: CD3$^-$CD16$^+$ CD56$^+$ NK / µl in different time points post allogeneic HSCT (Mean, Minimal-/ Maximal values)

Time post HSCT	Mean	Minimum	Maximum
Day 30	266	37	592
Day 60	273	37	600
Day 90	314	46	610
Day 120	301	56	590
Day 150	289	55	536
Day 180	337	76	918
Day 210	292	60	915
Day 240	302	76	920
Day 270	344	48	945
Day 300	355	56	963
Day 330	370	76	981
Day 360	400	82	985

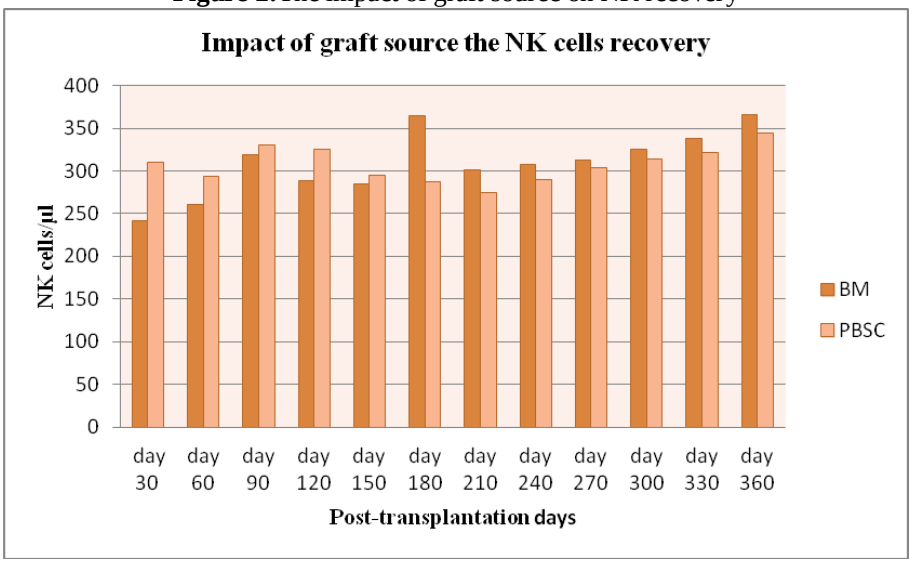
Figure 1: Recovery of CD3^{-}CD16^{+} CD56^{+} NK / µl



Impact of graft source on the recovery of NK cells:

Upon the correlation with the factors that affect the regeneration of NK cells after allogeneic haematopoitic stem cell transplantation, we had compared the impact of graft source on the kinetics of Natural killer cells recovey.The recovery of NK cells was better in PBSC reciepients between day 30 and day 150(P≤ 0.001) After day 180 ,the regeneration of natural killer cells occured faster in bone marrow reciepients(P≤ 0.009).

Figure 2: The impact of graft sourec on NK recovery



Impact of chronic GVHD (cGVHD) on the recovery of NK cells:

The reconstitution of NK cells over time was significantly higher in patients without chronic graft versus host disesae) than in those with symptoms of chronic graft versus host (P= 0.009).

Figure 3: The impact of chronic graft versus host disease on NK recovery

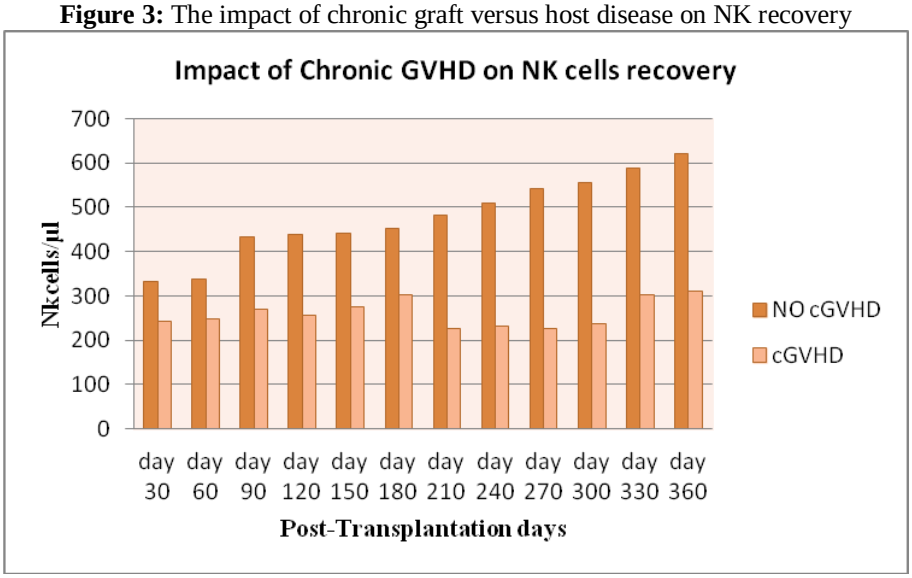


Table 3: Shows Factors influencing NK cells recovery

NK cells recovery	P value
Reconstitution day 90	≤ 0.002
Reconstitution day 180	≤ 0.001
Reconstitution day 270 to 360	≤ 0.009
Graft source: PBSC versus BM between day 30 to day 150	≤ 0.001
Graft source: PBSC versus BM after day 180	≤ 0.009
Chronic GVHD: no versus yes	0.009

IV. DISCUSSION

According to our results, Natural killer cell early reconstitution occurs early and stabilized from and 9 to 12 months after-transplantation; however, there are periods when the absolute count is noted to be lower, and many reasons such as opportunistic infections and graft versus host disease (GVHD) had a negative impact on the NK cell recovery.

NK cell recovery in recipients of allogeneic HSCT has been observed to start in the first two months [14, 15] but it can be delayed for up to six months in patients suffering from infections, especially fungal infections within first hundred days after transplantation [16].

In analyzed patients, the absolute count of natural killer cells were reduced in certain periods such as between day 90 and day 180 and after day 180 until day 270 following transplantation. That can be as result of the effect of immunosuppressive treatment that used for GVHD therapy.

According to the literature, within the first periods after allogeneic HSCT, the number of NK cells should be > 0.75 x 10^8/L when the source of graft is the bone marrow [17].

According to the influence of graft source on the kinetics of Natural killer cells recovey, there was a rapid recovery of NK cells in PBSC reciepients more than those of bone marrow between the first month and fifth month post HSCT. After six months ,the regeneration of natural killer cells was more rapidly in bone marroe reciepients [18].

Depending on our observatios in the analyzed patients, the regeneration of NK cells was lower in patients with chronic graft versus host disesae due to the negative impact of immunosuppressive treatment that used for GVHD therapy on NK reconstitution.

To summarize our results, the recovery of natural killer cells occurs early after allogeneic transplantation and there are periods when the numbers of NK cells were lower particularly between three and six months and between six and ninth month. The NK cells regeneration was better in patients without chronic graft versus host disease.

There are different external factors that have an influence on the recovery of NK cells in our patients:

-The underlying disease which HSCT is carried out for.

- The conditioning regimen and the use of immunosuppressive treatment early post transplantation as a preventive therapy for acute GVHD generates a decrease in lymphopoiesis and therefore, NK cell recovery was affected during the first three months post transplantation.

-Type of transplantation.

The recovery of NK cells in some periods was deficient and transient in some patients, probably due to NK cells sequestration at the infection target site or to the cell migration to the specific GVHD tissues (liver and skin). The rapid reconstitution of NK cells was observed in the period between three and six months after transplantation, from which it is understood that there was delayed NK cell regeneration in our patients, considering data reported in the literature.

There are two factors that have directly impact on the delay of immune recovery post HSCT:

-Slow de novo generation of donor-derived immune cells [19, 20].

- Cell and tissue damage due to effect of the conditioning regimen.

In this study, natural killer cells absolute counts were analyzed; however, immune system balance also needs their functional recovery, since NK cell function can be helpful in the production of antibodies, cytokines or cytotoxic ability even without the values measured in healthy subjects being reached.

In HSCT patients, who are immunosuppressed, NK cells have an essential role in the defence against opportunistic infections such as viral infections since they are the first line of immune defence, and their recovery is critical for the transplantation success [21].

In this study, we have found a relationship between the low frequencies of NK cells and chronic graft-versus-host disease (GVHD). Frequencies of CD3^{-} CD16^{+} CD56^{+} NK cells were significantly lower in patients with symptoms of chronic GVHD. After six months of transplantation, the recovery of NK cells was better in bone marrow recipients.

V. CONCLUSION

Natural killer cells have an important role in the natural immunity against infection and malignancy and the knowledge of their receptors and functions is helpful for the development of new treatment strategies in malignancy therapy.

There are different factors that have impact on NK cells reconstitution post haematopoietic stem cell transplantation (HSCT). We carried out an observing study of NK cell reconstitution in 20 -HSCT recipients during the first twelve months post transplantation.

In this work, we had described the regeneration of NK cells after allogeneic hematopoietic stem cell transplantation, and discussed the effect of graft source and chronic graft versus host disease on their retrieval.

The estimation of the role of the recovery of natural killer cells after allogeneic hematopoietic stem cell transplantation may aid in the development of new therapeutic measurement that may increase the chance of HSCT success.

REFERENCES

[1]. Ruggeri L, Mancusi A, Capanni M, et al. Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. Blood. (2007) 110:433-440.

[2]. Lanier LL, Phillips JH, Hackett J, et al. Natural killer cells: definition of a cell type rather than a function. J Immunol. (1986) 137: 2735-2739.

[3]. Vivier E, Ugolini S. Natural killer cells: from basic research to treatments. Front Immuno*l.* (2011) 2: 18.

[4]. Moretta A, Marcenaro E, Parolini S, et al. NK cells at the interface between innate and adaptive immunity. Cell Death Differ. (2008) 15: 226-233.

[5]. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol. (2008) 9: 495-502.

[6]. Trinchieri G. Biology of natural killer cells. Adv Immuno*l.*(1989) 47:187-376

[7]. Huenecke S, Cappel C, Esser R, et al. Development of three different NK cell subpopulations during immune reconstitution after paediatric allogeneic hematopoietic stem cell transplantation: prognostic markers in GvHD and viral infections. Front Immunol *.(2017) 8: 109*

[8]. Hokland M, Jacobsen N, Ellegaard J, et al. Natural killer function following allogeneic bone marrow transplantation. Very early reemergence but strong dependence of cytomegalovirus infection. Transplantation. (1988) 45:1080-1084.

[9]. Bellone G, Valiante NM, Viale O, et al. Regulation of haematopoiesis in vitro by alloreactive natural killer cell clones. J Exp Med.(1993)177:1117-1125

[10]. Patarca R, Fletcher MA, Podack ER. In: Manual of clinical laboratory immunology Rose, Conway de macario, Folds, et al, eds. Washington Dc: American Society for Microbiology Press(1997) 96-303

[11]. Yokoyama WM. In: Fundamental Immunology. Paul, WE, ed. New York, NY: Lippincott-Raven (1999)575-603**.**

[12]. Murphy WJ, Koh CY, Raziuddin A, et al. Immunobiology of natural killer cells and bone marrow transplantation: merging of basic and preclinical studies. Immunol Rev. (2001) 181:279-289

[13]. Ruggeri L, Capanni M, Casucci M, et al. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem-cell transplantation. Blood.(1999) 94:333-339

[14]. Saliaba RM, Rezvani K, Leen A, et al. General and virus-specific immune cell reconstitution after double cord blood transplantation. Biol Blood Marrow Transplant. (2015) 21:1284-1290.

[15]. Petersen SL, Ryder LP, Björk P, et al. A comparison of T-, B- and NK-cell reconstitution following conventional or non-myeloablative conditioning and transplantation with bone marrow or peripheral blood stem cells from human leukocyte antigen identical sibling donors. Bone Marrow Transplant. (2003) 32:65-72**.**

[16]. Orange JS. Human natural killer cell deficiencies. Curr Opin Allergy Clin Immunol. (2006) 6:339-409

[17]. De Koning C, Plantinga M, Besseling P, et al. Immune reconstitution after allogeneic hematopoietic cell transplantation in children. Biol Blood Marrow Transplant. (2016) 22:195-206

[18]. Eyrich M, Lang P, Lal S, et al. A prospective analysis of the pattern of immune reconstitution in a paediatric cohort following transplantation of positively selected human leukocyte antigen-disparate haematopoietic stem cell from parental donors. B*r* J Haematol. (2001) 114:422-432**.**

[19]. Huttunen P, Taskinen M, Siitonen S, et al. Impact of very early CD4$^+$/CD8$^+$ T cell counts on the occurrence of acute graft-versus-host disease and NK cell counts on outcome after paediatric allogeneic hematopoietic stem cell transplantation. Pediatr Blood Cancer. (2015) 62:522-528.

[20]. Jacobs R, Stoll M, Stratmann G, et al. CD16- CD 56+ natural killer cells after bone marrow transplantation. Blood. (1992) 79:3239-3244.

[21]. Kheav VD, Busson M, Scieux C, et al. Favourable impact of natural killer cell reconstitution on chronic graft-versus-host disease and cytomegalovirus reactivation after allogeneic hematopoietic stem cell transplantation. Haematologica. (2014) 99:1860-1867.

.