



# HOKKAIDO UNIVERSITY

Title	Hypogammaglobulinemia with a selective delayed recovery in memory B cells and an impaired isotype expression after rituximab administration as an adjuvant to autologous stem cell transplantation for non-Hodgkin Lymphoma
Author(s)	Nishio, Mitsufumi; Fujimoto, Katsuya; Yamamoto, Satoshi et al.
Citation	European Journal Of Haematology, 77(3), 226-232 <a href="https://doi.org/10.1111/j.1600-0609.2006.00693.x">https://doi.org/10.1111/j.1600-0609.2006.00693.x</a>
Issue Date	2006-09
Doc URL	<a href="https://hdl.handle.net/2115/28742">https://hdl.handle.net/2115/28742</a>
Rights	The definitive version is available at <a href="http://www.blackwell-synergy.com">www.blackwell-synergy.com</a>
Type	journal article
File Information	EJH77-3.pdf



**Hypogammaglobulinemia with a selective delayed recovery in memory B cells and an impaired isotype expression after rituximab administration as an adjuvant to autologous stem cell transplantation for non-Hodgkin lymphoma**

Mitsufumi Nishio<sup>1</sup>, Katsuya Fujimoto<sup>1</sup>, Satoshi Yamamoto<sup>1</sup>, Tomoyuki Endo<sup>1</sup>, Toshiya Sakai<sup>1</sup>, Masato Obara<sup>1</sup>, Kohki Kumano<sup>1</sup>, Koichiro Minauchi<sup>1</sup>, Keisuke Yamaguchi<sup>1</sup>, Yukari Takeda<sup>1</sup>, Norihiro Sato<sup>2</sup>, Kazuki Koizumi<sup>3</sup>, Masaya Mukai<sup>3</sup> and Takao Koike<sup>1</sup>

From the <sup>1</sup>Department of Medicine II, Hokkaido University Graduate School of Medicine, Sapporo, Japan; <sup>2</sup>Transfusion Service, Hokkaido University Hospital, Sapporo, Japan; <sup>3</sup>Sapporo City General Hospital, Sapporo, Japan

**Corresponding Author:** Dr. Mitsufumi Nishio

N15 W7, Kita-ku, Sapporo, 060-8638, Japan

Phone: 81-11-706-5915, FAX 81-11-706-7710, e-mail m-nishio@med.hokudai.ac.jp

**Running Title: APBSCT/rituximab and hypogammaglobulin**

**Abstract Word Count: 235**

**Text Word Count (excluding abstract): 2,742**

**Number of Figure: 1**

**Number of Tables: 4**

Key Words: Rituximab, APBSCT, hypogammaglobulinemia, memory B-cell, CVID

## Abstract

**Objectives:** Some studies have indicated patients who received rituximab as adjuvant to stem cell transplantation had an increased risk of developing severe hypogammaglobulinemia. The mechanism of this hypogammaglobulinemia is unknown, although investigators have hypothesized a further delay in the B-cell recovery as one potential etiology. The aim of this study is to clarify the mechanism(s) of this hypogammaglobulinemia.

**Methods:** A total of fourteen patients with high-risk CD20<sup>+</sup> lymphoma underwent an autologous peripheral blood stem cell transplantation (APBSCT). After a hematological recovery, rituximab was given weekly for up to four doses as an adjuvant therapy.

**Results:** After a median follow-up of 33.5 months, we found six patients (Group A) who had hypogammaglobulinemia, while the eight other patients (Group B) had normal serum immunoglobulin levels. A phenotypical analysis revealed that Group A patients had already achieved B-cell recovery. However, we found a severe delay in the recovery of CD27<sup>+</sup> memory B cells, especially in the IgD<sup>-</sup>/CD27<sup>+</sup> switched populations in Group A, but CD27 negative naive B-cells reverted to a normal range in both groups. Consistent with this, RT-PCR studies with peripheral blood mononuclear cells revealed that most patients in Group A lacked more than two classes of isotype transcripts.

**Conclusions:** Abnormal repertoires and impaired isotype expression are seen in patients with common variable immunodeficiency, these data suggested that rituximab after APBSCT can affect not only the B-cell quantities, but also the recovery of the B-cell repertoires.

## Introduction

High dose chemotherapy followed by autologous peripheral blood stem cell transplantation (APBSCT) has been widely used for patients with non-Hodgkin's lymphoma (NHL) (1-3). Although APBSCT for patients with high risk aggressive lymphoma may give a prolonged disease free survival (4), relapse is still the primary cause of failure after APBSCT. In addition to residual disease after APBSCT, the reinfusion of tumor cells in the autologous graft may contribute to a relapse. Rituximab, a chimeric anti-CD20 monoclonal antibody, has a good safety profile and, hence, it has been incorporated into many chemotherapeutic regimens and in vivo purging before autologous graft harvest (5, 6). It has been also used as one of the most effective approaches for adjuvant therapy after APBSCT to eradicate the minimal residual disease that leads to relapse (7-9).

Rituximab alone does not appear to cause severe hypogammaglobulinemia according to initial phase I and II clinical trials (10). However, recent studies revealed that patients who received rituximab as an adjuvant to APBSCT had an increased risk of developing severe hypogammaglobulinemia (7, 8, 11). In addition, prolonged hypogammaglobulinemia with rituximab has also been seen in patients with post transplant Epstein-Barr virus associated lymphoproliferative disorder (EBLPD) (12-15) or human immunodeficiency virus (HIV) associated lymphoma (16). The mechanism of this hypogammaglobulinemia is unknown, although investigators have hypothesized a delay in the B-cell recovery as one potential etiology.

We previously reported severe persistent panhypogammaglobulinemia after rituximab therapy for post transplant Epstein-Barr virus associated autoimmune hemolytic anemia in a Japanese woman (17). We found her B cells to be composed of only CD27 negative naive B cells. In addition, her peripheral blood

mononuclear cells (PBMC) only expressed transcriptions of IgM, IgG1, IgA1 and IgA2 but no IgG2, IgG3 and IgG4. These impaired B-cell repertoires, which are also seen in patients with common variable immunodeficiency (CVID) (18), were speculated to cause her hypogammaglobulinemia. We hypothesized that the same abnormal patterns could be found in the NHL patients treated with APBSCT plus rituximab and therefore performed phenotypical and genetic studies to test our hypothesis.

## **Patients, materials and methods**

### ***Patients and Treatment***

From August 2001 to August 2004, a total of 17 Japanese patients with newly diagnosed B-cell NHL were enrolled in our protocol which included APBSCT (5, 19). The eligibility criteria for APBSCT were as follows: age between 15 and 65 years; a confirmed histologic diagnosis of CD20-positive B-cell NHL (excluding Burkitt's lymphoma and lymphoblastic lymphoma); prognostic index, constructed by Coiffier et al (20), of intermediate or high risk as defined by having at least one of the four following adverse features: (1) elevated serum lactic dehydrogenase (LDH) level, (2) bulky mass >10 cm, (3) advanced stage III or IV according to Ann Arbor staging classification, (4) two or more extranodal sites: no severe cardiopulmonary, renal and hepatic dysfunction. After APBSCT, six patients, two who died of NHL and four who were seen by the local physicians, dropped out during the follow-up. Besides these eleven patients, three patients (UPN4, 9 and 13) were enrolled during their first relapse after three to six cycles of CHOP. As a result, a total of fourteen patients were evaluated in this study.

All newly diagnosed patients were initially treated with three cycles of CHOP (21) every two weeks with G-CSF (Lenograstim, Chugai Pharmaceutical CO., Tokyo, Japan) support as induction chemotherapy. One or two leukapheresis were performed after a fourth cycle of CHOP therapy (5). After November 2001, when rituximab was available in Japan, PBSC were mobilized using CHOP plus rituximab (Chugai Pharmaceutical CO., Tokyo, Japan) intended as in vivo purging (5). After the fourth cycle of CHOP, the patients' responses to the initial CHOP were evaluated. While those who achieved tumor reduction rates of more than 50% were treated with two additional cycles of CHOP, those showing less than a 50% response were

considered to be resistant to CHOP and thus were treated with other salvage regimens, including two cycles of DHAP (dexamethasone, cytarabine and cisplatin) (22) or FMD (fludarabine, mitoxantrone and dexamethasone) regimens (23). Pre-transplant conditioning regimens for all patients consisted of ranimustine, carboplatin, etoposide and cyclophosphamide (MCVC regimen) (19). After engraftment, rituximab at a dose of 375 mg/m<sup>2</sup> was given weekly for up to four weeks. All patients achieved complete remission after APBSCT. The approval to perform these studies was obtained from the Hokkaido University Hospital's and the Sapporo City General Hospital's institutional review board. Informed consent was provided according to the Declaration of Helsinki protocol.

### ***Cell Preparation***

After obtaining the patients' informed consent, blood samples were collected from the patients at the Hokkaido University Hospital with the approval of the ethics committee of Hokkaido University. Peripheral blood mononuclear cells (PBMC) were isolated via density-gradient centrifugation with Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). Normal PBMC were obtained from the buffy-coat of blood samples collected from adult volunteers at the Hokkaido Blood Bank.

### ***Flow Cytometry***

Three-color flow cytometric immunophenotyping was performed to determine the proportion of lymphocyte subsets. The following monoclonal antibodies specific for human surface antigens were used: anti-CD19-phycoerythrin (PE)-Cy5, anti-CD27-PE, anti-IgD-fluorescein isothiocyanate (FITC), anti-CD3-FITC, anti-CD4-FITC, anti-CD8-PE and anti-CD56-PE were from BD PharMingen (San Diego, CA), and the

appropriate isotype controls from DAKO Japan, (Kyoto, Japan). The cells were stained with saturating amounts of antibodies for 30 minutes at 4° C in Deficient RPMI-1640 (Sigma Aldrich, St. Louis, MO) supplemented with 0.5% bovine serum albumin, washed 2 times, and then were analyzed on a FACSCalibur (Becton Dickinson, Mountain View, CA). Flow cytometry data were analyzed using CellQuest™ software (Beckton Dickinson, San Jose, CA). A minimum 50,000 cells per sample were acquired on a flow cytometer.

### ***RT-PCR of immunoglobulin transcripts***

Total RNA was isolated from PBMC ( $5 \times 10^6$  cells), using the RNeasy Mini Kit (QIAGEN, Valencia, CA). Subsequently, first-strand cDNA synthesis was performed with SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. The primers of the different human immunoglobulin isotypes were used as described previously (18). PCR amplification was performed with reverse-transcribed material and 0.5 U AmpliTaq Gold polymerase (Takara, Japan) with an initial step of 5 min at 95°C; then 40 cycles each consisting of 1 min of extension at 72°C; and at least a final extension for 10 min at 72°C. The reaction products were electrophoresed on 2% agarose gel.

### ***Statistical Analysis***

For statistical comparisons between the groups, the Mann-Whitney U-test was used. Analyses were performed using Glanzman's "Primer of Biostatistics" software package (McGraw-Hill Inc., New York, NY).

## Results

### ***Incidence of hypogammaglobulinemia after APBSCT and rituximab***

After a median follow-up of 33.5 months (range 12 to 43 months), we found six patients who had hypogammaglobulinemia (Group A), as defined by the serum IgG levels of less than 8.0 g/l (normal range 8.7 to 17.0 g/l), while the other eight patients (Group B) had normal serum IgG levels. There were three follicular lymphoma (FL), two diffuse large cell lymphoma (DLCL) and one mantle cell lymphoma in Group A, and three FL and five DLCL in Group B. The backgrounds of the two groups were not different, including age, the number of CD34 positive cells transplanted, the follow-up period or the dose of rituximab pre- or post-APBSCT (Table 1). We also found the IgA levels to be significantly lower in Group A than Group B ( $0.46\pm 0.27$  g/l and  $1.55\pm 0.58$  g/l, mean $\pm$ S.D.,  $p<0.01$ ), as well as the IgG levels ( $5.89\pm 1.72$  g/l and  $10.9\pm 2.60$  g/l) but that was not the case for IgM ( $0.50\pm 0.39$  g/l and  $0.85\pm 0.26$  g/l).

### ***Absence of memory B cells repertoires in patients who have hypogammaglobulinemia***

Since hypogammaglobulinemia after APBSCT and rituximab therapy had been hypothesized to arise due to the delayed B-cell recovery, we evaluated the number of B-cells in eleven patients. Unexpectedly, both Group A and Group B patients had already achieved a B-cell recovery to a normal (Group A  $135\pm 104 \times 10^6/l$  and Group B  $235\pm 113 \times 10^6/l$ , mean $\pm$ S.D., *N.S.*). Recent studies have shown the CD27 positive memory B-cells to be defective, especially IgD negative switched populations, in some patients with CVID (18). As most patients with CVID have a normal number of B-cells, we hypothesized that the hypogammaglobulinemia seen

after APBSCT and rituximab may therefore be caused by the same mechanism(s) as CVID. To test this hypothesis, we performed a detailed phenotypical analysis on these eleven patients by flow cytometry.

While CD27 negative naive B-cell populations were evident with flow cytometric analysis, CD27<sup>+</sup> memory B-cell populations were hardly seen in the PBMC from Group A (Figure 1). The absolute numbers of IgD<sup>-</sup>/CD27<sup>+</sup> switched memory B cells were as low as  $1 \pm 0 \times 10^6/l$ , but those of Group B were  $15 \pm 10 \times 10^6/l$  (mean $\pm$ S.D.,  $p < 0.01$ , Table 2). The recovery of CD27<sup>+</sup>/IgD<sup>+</sup> nonswitched memory B cells in Group A was also delayed as well (Group A  $2 \pm 1 \times 10^6/l$  and Group B  $8 \pm 6 \times 10^6/l$ , Mean $\pm$ SD,  $p < 0.05$ , Table 2), but the number of CD27 negative naive B cells (Group A  $130 \pm 102 \times 10^6/l$  and Group B  $205 \pm 103 \times 10^6/l$ , mean $\pm$ S.D.) recovered to a normal range in both groups (Table 2). In contrast to the B-cell subset, the number and ratio of either CD4<sup>+</sup> or CD8<sup>+</sup> T cells and CD56<sup>+</sup> NK cells did not differ between the two groups (data not shown).

### ***Impaired isotype expression in patients who have hypogammaglobulinemia***

The phenotypical analysis suggested that Group A patients had similar abnormal patterns to those with CVID. As a result, we evaluated immunoglobulin isotype production by analyzing the immunoglobulin transcripts by RT-PCR, as patients with CVID are also known to have impaired immunoglobulin class switching (18, 24). Although control donors always expressed each isotype (data not shown), we observed that all patients tested in Group A had defected one or more isotype mRNA transcript expressions in IgG (Table 3). On the other hand, all patients in Group B, except for UPN12 who lacked only IgG4 transcript, showed the normal IgG transcript with RT-PCR. While the IgM mRNA transcript was seen in PBMC of all

patients, the transcript of IgA2 was absent in six out of thirteen tested patients and there appeared to be no differences between the two groups. Taken together with the phenotypical analysis, these data indicate that Group A patients had not only a small number of B-cells, but also abnormal repertoires in B-cells.

### ***Bone marrow examinations after APBSCT and rituximab***

Bone marrow examinations were performed in nine patients to evaluate any evidence of a relapse. A total 500 cells were evaluated microscopically, and the plasma cells were recognized by their unique morphology. While we found plasma cells (normal range 0.8-1.6%) in all five specimens tested in the Group B patients, only one out of four from Group A specimens showed the existence of plasma cells in the bone marrow. (Table 4)

### ***Clinical complications with hypogammaglobulinemia***

UPN1 developed repeated bacterial infections and required the intravenous administrations of immunoglobulin (IVIG). However, the other five patients in Group A, as well as all patients in Group B, have been asymptomatic and required no IVIG.

## Discussion

Increasingly, attention is being focused on immune-based therapy for the treatment of NHL. Rituximab is the one of the most frequently administered and well-established monoclonal antibodies which specifically targets CD20 antigen on malignant B cells. CD20 is also expressed on normal B cells, but not on plasma cells, and that has been believed to be the reason why this monoclonal antibody would not cause hypogammaglobulinemia when it was administered as a single agent in earlier studies. However, our survey revealed that six out of fourteen patients (43%) developed hypogammaglobulinemia which was much higher than expected in light of its safety profile when it is used as a single agent.

It has recently been established that CD27 expression could allow for the identification of functional human B cells (25). The impaired B cell subpopulation was noticed in CVID. Piqueras et al proposed a classification based on the quantitative repartition of naive/memory B cells according to the dual expression of IgD and CD27 (18). In their classification, the numbers of IgD<sup>+</sup>/CD27<sup>-</sup> naive B cells were not statistically different from those seen in healthy donors, but CD27 positive memory B cells, especially IgD<sup>-</sup>/CD27<sup>+</sup> switched memory B cells were significantly decreased. In our cohort, only Group A patients showed the same abnormal patterns of B cells repertoires seen in CVID patients (Figure 1 and Table 2).

In addition, the defects of some isotype transcripts in PBMC, which have also been found in patients with CVID, were dominantly seen in Group A (Table 3). But this defect of isotype expression was not specific for Group A, as some patients in Group B also lacked transcripts, especially in IgA2 class. At this moment, it is still unclear as to why the patients in Group A had significantly lower values of IgA than Group B. On the other hand, the transcripts of IgM were evident in all patients.

This suggests that the development of B cells before class switching were maintained in Group A patients. This fact was compatible with the fact that B cells of Group A were composed of mainly naive B cells, which were in the developmental stage before a somatic hypermutation in the germinal center.

Horowitz and colleagues (8) noticed that median IgG levels remained suppressed at less than 10 g/l at 24 months when absolute B-cell number recovered to the normal range in 14 out of 20 patients treated with APBSCT and rituximab. Goldberg et al (26) reported the unusual viral infections in four patients with severe hypogammabulinemia who were treated with APBSCT and peritransplant rituximab. One patient had a normal number of B-cells when she developed various infections. Since both studies simply showed the whole B-cell counts, whether the same abnormal patterns of B-cells existed in Group A remained unclear. However, it is evident that a small number of B cells could not account for all pathogenesises of hypogammaglobulinemia after APBSCT and rituximab therapy. On the other hand, Lim et al (11) reported hypogammaglobulinemia, especially in IgM, in patients administered rituximab after APBSCT every 3 months throughout 2-year period. They speculated that a continuous B-cell depletion was the main cause of the severely delayed immunoglobulin recovery. Horowitz and we found a severe reduction of IgG (8) instead of IgM. The schedule of rituximab after APBSCT might therefore influence the class of hypogammaglobulinemia.

The reported cases with rituximab related hypogammaglobulinemia were mostly seen in patients who were put under the additional cellular immunosuppression, namely stem cell transplantations (7, 8, 26), organ transplantations (12-14) or HIV infection (16). Moreover, the maintenance rituximab after allogeneic stem cell transplantation was complicated with

hypogammaglobulinemia more often than autologoustransplantation (7). In contrast to Group A patients, we did not see any hypogammaglobulinemia or abnormal B-cell repertoires in five patients who underwent allogeneic transplantation without rituximab in our institution (data not shown). It is believed that allogeneic stem cell transplantation causes patients to demonstrate a more profound immunosuppressive status for prophylaxis of graft versus host disease. However, a simple immunosuppressive therapy without rituximab did not appear to induce the hypogammaglobulinemia with an abnormal repertoire of B cells. This suggests that rituximab played a central role in the imbalanced B-cell recovery. In a normal B cell system, naive B cells that stimulated by surrogate surface B cell receptors with the support of CD40 ligand from T cells differentiate into germinal center B cells, then after somatic hypermutations, they finally become memory B cells or long lived plasma cells. The interaction of these T cells and/or antigen presenting cells and B cells might thus play a role in the pathogenesis of persistence hypogammaglobulinemia. Another possible mechanism could be the blocking of maturation capacity in naive B cells which could cause a persistent depletion of the plasma cell populations in bone marrow. This was supported by the persistent depletion of plasma cells in most patients in Group A (Table 4). However, more cases are required to confirm an absence of plasma cells to determine the precise pathogenesis of this hypogammaglobulinemia.

In conclusion, our results suggest that rituximab administration after APBSCT cause hypogammaglobulinemia. Although only one patient was symptomatic in our cohort, clinicians should pay extra attention for the possible risk of infections in this immunocompromised situation after high dose chemotherapy. Whether this complication is reversible or not needs to be determined by a longer follow-up. In

addition, whether rituximab administration with less intensive chemotherapy, such as CHOP, can induce hypogammaglobulinemia with abnormal B-cell repertoires should in the future be explored in patients who received rituximab without APBSCT.

## **Acknowledgement**

The authors are grateful to Ikumi Sato for technical assistance and Brian Quinn for language assistance.

## Figure Legends

### Figure 1

**Selective loss of IgD<sup>-</sup>/CD27<sup>+</sup> switched memory B cells after treatment with APBSCT and rituximab.**

Peripheral blood mononuclear cells were stained with IgD-FITC, CD27-PE and CD19-PECy5. Naive and memory B cells were defined by membrane expressing IgD and CD27 markers with gating on CD19<sup>+</sup> B cells.

## References

1. Strehl J, Mey U, Glasmacher A, Djulbegovic B, Mayr C, Gorschluter M, Ziske C, Schmidt-Wolf IG. High-dose chemotherapy followed by autologous stem cell transplantation as first-line therapy in aggressive non-Hodgkin's lymphoma: a meta-analysis. *Haematologica* 2003;88:1304-1315.
2. Santini G, Salvagno L, Leoni P, Chisesi T, De Souza C, Sertoli MR, Rubagotti A, Congiu AM, Centurioni R, Olivieri A, Tedeschi L, Vespignani M, Nati S, Soracco M, Porcellini A, Contu A, Guarnaccia C, Pescosta N, Majolino I, Spriano M, Vimercati R, Rossi E, Zambaldi G, Mangoni L, Rizzoli V. VACOP-B versus VACOP-B plus autologous bone marrow transplantation for advanced diffuse non-Hodgkin's lymphoma: results of a prospective randomized trial by the non-Hodgkin's Lymphoma Cooperative Study Group. *J Clin Oncol* 1998;16:2796-2802.
3. Haioun C, Lepage E, Gisselbrecht C, Bastion Y, Coiffier B, Brice P, Bosly A, Dupriez B, Nouvel C, Tilly H, Lederlin P, Biron P, Briere J, Gaulard P, Reyes F. Benefit of autologous bone marrow transplantation over sequential chemotherapy in poor-risk aggressive non-Hodgkin's lymphoma: updated results of the prospective study LNH87-2. Groupe d'Etude des Lymphomes de l'Adulte. *J Clin Oncol* 1997;15:1131-1137.
4. Shipp MA, Abeloff MD, Antman KH, Carroll G, Hagenbeek A, Loeffler M, Montserrat E, Radford JA, Salles G, Schmitz N, Symann M, Armitage JO, Philip T, Coiffier B. International Consensus Conference on High-Dose Therapy with Hematopoietic Stem Cell Transplantation in Aggressive Non-Hodgkin's Lymphomas: report of the jury. *J Clin Oncol* 1999;17:423-429.
5. Endo T, Sato N, Mogi Y, Koizumi K, Nishio M, Fujimoto K, Sakai T, Kumano K, Obara M, Ikeda H, Koike T. Peripheral blood stem cell mobilization following

CHOP plus rituximab therapy combined with G-CSF in patients with B-cell non-Hodgkin's lymphoma. *Bone Marrow Transplant* 2004;33:703-707.

6. Arcaini L, Orlandi E, Alessandrino EP, Iacona I, Brusamolino E, Bonfichi M, Bernasconi P, Calatroni S, Tenore A, Montanari F, Troletti D, Pascutto C, Regazzi M, Lazzarino M. A model of in vivo purging with Rituximab and high-dose AraC in follicular and mantle cell lymphoma. *Bone Marrow Transplant* 2004;34:175-179.

7. Shimoni A, Hardan I, Avigdor A, Yeshurun M, Raanani P, Ben-Bassat I, Nagler A. Rituximab reduces relapse risk after allogeneic and autologous stem cell transplantation in patients with high-risk aggressive non-Hodgkin's lymphoma. *Br J Haematol* 2003;122:457-464.

8. Horwitz SM, Negrin RS, Blume KG, Breslin S, Stuart MJ, Stockerl-Goldstein KE, Johnston LJ, Wong RM, Shizuru JA, Horning SJ. Rituximab as adjuvant to high-dose therapy and autologous hematopoietic cell transplantation for aggressive non-Hodgkin lymphoma. *Blood* 2004;103:777-783.

9. Brugger W, Hirsch J, Grunebach F, Repp R, Brossart P, Vogel W, Kopp HG, Manz MG, Bitze M, Schlimok G, Kaufmann M, Ganser A, Fehnle K, Gramatzki M, Kanz L. Rituximab consolidation after high-dose chemotherapy and autologous blood stem cell transplantation in follicular and mantle cell lymphoma: a prospective, multicenter phase II study. *Ann Oncol* 2004;15:1691-1698.

10. Maloney DG, Grillo-Lopez AJ, Bodkin DJ, White CA, Liles TM, Royston I, Varns C, Rosenberg J, Levy R. IDEC-C2B8: results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. *J Clin Oncol* 1997;15:3266-3274.

11. Lim SH, Zhang Y, Wang Z, Esler WV, Beggs D, Pruitt B, Hancock P, Townsend M. Maintenance rituximab after autologous stem cell transplant for high-

risk B-cell lymphoma induces prolonged and severe hypogammaglobulinemia. *Bone Marrow Transplant* 2005;35:207-208.

12. Verschuuren EA, Stevens SJ, van Imhoff GW, Middeldorp JM, de Boer C, Koeter G, The TH, van Der Bij W. Treatment of posttransplant lymphoproliferative disease with rituximab: the remission, the relapse, and the complication. *Transplantation* 2002;73:100-104.

13. Kuehnle I, Huls MH, Liu Z, Semmelmann M, Krance RA, Brenner MK, Rooney CM, Heslop HE. CD20 monoclonal antibody (rituximab) for therapy of Epstein-Barr virus lymphoma after hemopoietic stem-cell transplantation. *Blood* 2000;95:1502-1505.

14. Imashuku S, Teramura T, Morimoto A, Naya M, Kuroda H. Prolonged hypogammaglobulinemia following rituximab treatment for post transplant Epstein-Barr virus-associated lymphoproliferative disease. *Bone Marrow Transplant* 2004;33:129-130.

15. Castagnola E, Dallorso S, Faraci M, Morreale G, Di Martino D, Cristina E, Scarso L, Lanino E. Long-lasting hypogammaglobulinemia following rituximab administration for Epstein-Barr virus-related post-transplant lymphoproliferative disease preemptive therapy. *J Hematother Stem Cell Res* 2003;12:9-10.

16. Miles SA, McGratten M. Persistent panhypogammaglobulinemia after CHOP-rituximab for HIV-related lymphoma. *J Clin Oncol* 2005;23:247-248.

17. Nishio M, Endo T, Fujimoto K, Sato N, Sakai T, Obara M, Kumano K, Minauchi K, Koike T. Persistent panhypogammaglobulinemia with selected loss of memory B cells and impaired isotype expression after rituximab therapy for post-transplant EBV-associated autoimmune hemolytic anemia. *Eur J Haematol* 2005;75:527-529.

18. Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, Debre P, Schmitt C, Oksenhendler E. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. *J Clin Immunol* 2003;23:385-400.
19. Tarumi T, Sawada K, Koizumi K, Takano H, Fukada Y, Nishio M, Fujie T, Ohnishi K, Kohno M, Sato N, Sekiguchi S, Koike T. A pilot study of a response oriented chemotherapeutic regimen combined with autologous peripheral blood progenitor cell transplantation in aggressive non-Hodgkin's lymphoma. *Leuk Lymphoma* 1999;34:361-371.
20. Coiffier B, Gisselbrecht C, Vose JM, Tilly H, Herbrecht R, Bosly A, Armitage JO. Prognostic factors in aggressive malignant lymphomas: description and validation of a prognostic index that could identify patients requiring a more intensive therapy. The Groupe d'Etudes des Lymphomes Agressifs. *J Clin Oncol* 1991;9:211-219.
21. McKelvey EM, Gottlieb JA, Wilson HE, Haut A, Talley RW, Stephens R, Lane M, Gamble JF, Jones SE, Grozea PN, Gutterman J, Coltman C, Moon TE. Hydroxyldaunomycin (Adriamycin) combination chemotherapy in malignant lymphoma. *Cancer* 1976;38:1484-1493.
22. Martelli M, Cimino G, Gastaldi R, Mantovani L, Sgadari C, Amadori S, Guglielmi C. DHAP treatment: results of 18 patients with refractory or recurrent non Hodgkin's lymphoma. *Haematologica* 1990;75:194-195.
23. Ma SY, Au WY, Chim CS, Lie AK, Lam CC, Tse E, Leung AY, Liang R, Kwong YL. Fludarabine, mitoxantrone and dexamethasone in the treatment of indolent B- and T-cell lymphoid malignancies in Chinese patients. *Br J Haematol* 2004;124:754-761.

24. Mori S, Kondo N, Motoyoshi F, Kaneko H, Inoue Y, Orii T. Diversity in DNA rearrangements and in RNA expressions of immunoglobulin gene on common variable immunodeficiency. *Eur J Immunogenet* 1992;19:273-285.
25. Agematsu K, Hokibara S, Nagumo H, Komiyama A. CD27: a memory B-cell marker. *Immunol Today* 2000;21:204-206.
26. Goldberg SL, Pecora AL, Alter RS, Kroll MS, Rowley SD, Waintraub SE, Imrit K, Preti RA. Unusual viral infections (progressive multifocal leukoencephalopathy and cytomegalovirus disease) after high-dose chemotherapy with autologous blood stem cell rescue and peritransplantation rituximab. *Blood* 2002;99:1486-1488.

Table 1 Patients' characteristics

UPN	Group A						Group B					
	1	2	3	4	5	6	7	8	9	10	11	
Age	65	59	45	50	49	64	63	57	56	44	51	
Sex	M	F	F	M	F	M	M	F	F	F	F	
Histology	MCL	FL	FL	FL	DLCL	DLCL	FL	DLCL	DLCL	FL	DLCL	
Clinical stage	IIA	IVA	IVA	IVA	IVA	IIIA	IVA	IIIA	IIIA	IVA	IV	
Months after APBSCT	42	39	36	17	15	12	43	39	39	34	31	
CD34+ cells transplanted (x10 <sup>6</sup> /kg)	2.8	5.1	1.9	3.5	3.2	3.2	2.5	3.0	4.3	4.0	3.0	
No. of rituximab administration												
Pre-APBSCT	0	1	1	4	6	7	0	0	0	2	2	
Post-APBSCT	4	3	3	4	2	1	4	4	4	2	2	
Cycles of chemotherapy before APBSCT	6	6	6	9	7	6	7	6	10	6	6	
Serum immunoglobulin levels												
IgA (g/l)	0.68	0.25	0.32	0.91	0.37	0.25	1.77	1.40	0.90	1.86	1.1	
IgG (g/l)	3.63	7.42	4.77	7.43	4.69	7.42	14.6	10.1	8.01	12.0	10	
IgM (g/l)	1.21	0.30	0.21	0.71	0.29	0.30	1.26	0.76	0.89	1.03	0.4	

UPN, unique patient number; M, male; F, female; MCL, mantle cell lymphoma; FL, follicular lymphoma; DLCL, diffuse large cell lymphoma; APBSCT, autologous peripheral blood stem cell transplantation

Table 2 B-cell subset reconstitution after APBSCT and rituximab

UPN	CD19 <sup>+</sup> (x10 <sup>6</sup> /l)	CD19 <sup>+</sup>		
		CD27 <sup>-</sup> (x10 <sup>6</sup> /l)	CD27 <sup>+</sup> /IgD <sup>+</sup> (x10 <sup>6</sup> /l)	CD27 <sup>+</sup> /IgD <sup>-</sup> (x10 <sup>6</sup> /l)
Group A				
1	131	128	2	1
2	84	81	1	1
3	72	65	3	1
4	334	324	3	2
5	141	138	2	1
6	47	44	2	1
Group B				
7	331	261	20	33
8	189	171	6	12
9	185	173	3	10
10	404	369	12	8
11	332	293	8	14
12	68	60	2	3
13	240	221	5	9
14	134	90	6	28

APBSCT, autologous peripheral blood stem cell transplantation; UPN, unique patient number

Table 3 Isotype expressions in peripheral blood mononuclear cells

UPN	IgM	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2
Group A							
1	+	-	+	-	+	+	+
2	+	+	+	-	+	+	-
3	+	+	-	+	+	+	-
4	ND	ND	ND	ND	ND	ND	ND
5	+	+	+	-	+	-	-
6	+	+	+	+	-	+	+
Group B							
7	+	+	+	+	+	+	-
8	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+
11	+	+	+	+	+	+	-
12	+	+	+	+	-	+	-
13	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+

UPN, unique patient number; ND, not done

Table 4 Bone marrow (BM) plasma cells proportions after APBSCT

UPN	% of BM plasm cells	Period of BM examinations after APBSCT
Group A		
1	ND	
2	0.4	14 months
3	0	24 months
4	0	12 months
5	0	6 months
6	ND	
Group B		
7	0.4	7 months
8	ND	
9	3.8	4 months
10	1.2	18 months
11	0.6	26 months
12	0.2	12 months
13	ND	
14	ND	

APBSCT, autologous peripheral blood stem cell transplantation;

UPN, unique patient number; ND, Not done

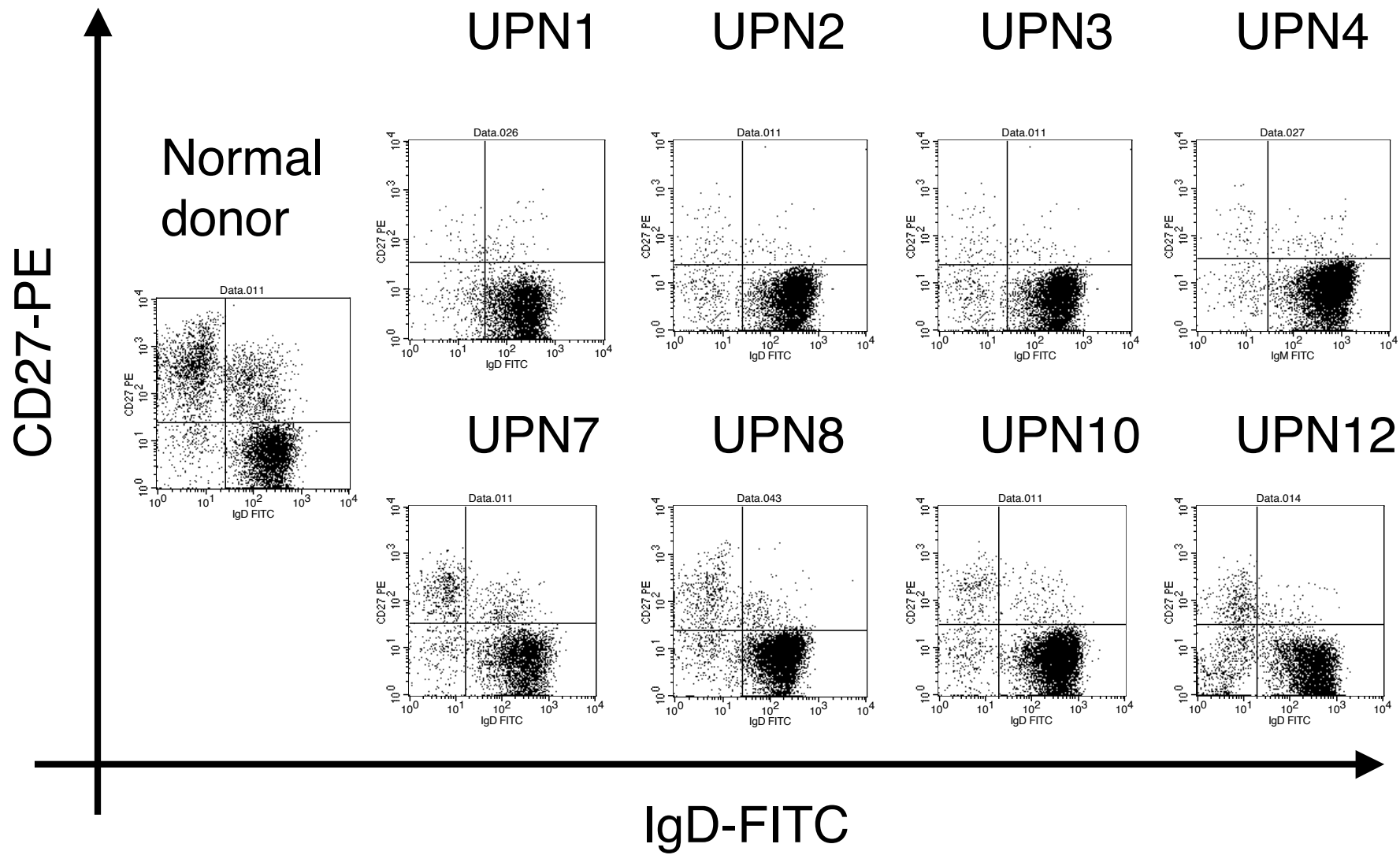


Figure 1