Prognostic significance of S-phase kinase-associated protein 2 and p27kip1 in patients with diffuse large B-cell lymphoma: effects of rituximab

R. Seki¹, K. Ohshima², T. Fujisaki³, N. Uike⁴, F. Kawano⁵, H. Gondo⁶, S. Makino⁷, T. Eto⁸, Y. Moriuchi⁹, F. Taguchi¹⁰, T. Kaminura¹¹, H. Tsuda¹², K. Shimoda¹³ & T. Okamura¹*

¹Division of Hematology and Oncology, Department of Medicine, Fukuoka; ²Department of Pathology, Research Center for Innovative Cancer Therapy, Kurume University School of Medicine, Fukuoka; ³Department of Internal Medicine, Matsuyama Red Cross Hospital, Ehime; ⁴Department of Hematology, National Kyushu Cancer Center, Fukuoka; ⁵Department of Internal Medicine, National Hospital Organization Kumamoto Medical Center, Kumamoto; ⁶Department of Internal Medicine, Saga Prefectural Hospital, Koseikan, Saga; ⁷Department of Internal Medicine, Miyazaki Prefectural Hospital, Miyazaki; ⁸Department of Hematology, Hamanomachi Hospital, Fukuoka; ⁹Department of Internal Medicine, Koseikan, Saga; ¹⁰Department of Internal Medicine, Miyazaki Prefectural Hospital, Miyazaki; ¹¹Department of Hematology, Hamamatsu Hospital, Fukuoka; ¹²Department of Medicine, Research Center for Innovative Cancer Therapy, Kurume; ¹³Department of Hematology, Faculty of Medicine, Miyazaki University, Miyazaki, Japan

Received 3 May 2009; revised 11 August 2009; accepted 14 September 2009

Background: The F-box protein S-phase kinase-associated protein 2 (Skp2) positively regulates the G1–S transition by promoting degradation of the cyclin-dependent kinase inhibitor p27kip1 (p27). Recent evidence has indicated an oncogenic role of Skp2 in not only carcinogenesis but also lymphomagenesis.

Materials and methods: Clinicopathologic features and immunohistochemical expression of Skp2 and p27 were studied retrospectively in 671 patients treated with cyclophosphamide, vincristine, doxorubicin and prednisolone (CHOP) or cyclophosphamide, vincristine, doxorubicin and prednisolone plus rituximab (R-CHOP). The median follow-up periods were 43.2 months in the CHOP group (n = 425) and 24.0 months in the R-CHOP group (n = 246).

Results: High Skp2 or low p27 expression correlated significantly with poor overall survival (OS) and progression-free survival (P < 0.001) in both treatment groups. The prognostic value of Skp2 or p27 expression was independent of the parameters included in the International Prognostic Index by multivariate analysis. Patients with high Skp2 expression in combination with low p27 expression showed the worst survival.

Conclusions: Addition of rituximab to the CHOP regimen did not provide a beneficial outcome to patients with diffuse large B-cell lymphoma with high Skp2 expression and low p27 expression. Skp2 and p27 may be useful prognostic markers in the rituximab era.

Key words: cell cycle, DLBCL, prognostic factor, p27, rituximab, Skp2

introduction

Diffuse large B-cell lymphoma (DLBCL) is one of the most common lymphoid neoplasms, characterized by heterogeneity in its clinical, immunophenotypic and genetic features [1, 2]. Recent studies have used gene expression profiling to identify the following three distinct subgroups of DLBCL: germinal center B-cell-like (GCB), activated B-cell-like and primary mediastinal (PM) DLBCL [2–4]. Several prognostic models on the basis of RNA or protein expression have been developed to predict survival in patients with DLBCL [5, 6]. However, a consensus on how to stratify patients with DLBCL has not been achieved. Many studies have also focused on immunohistochemistry as a method to identify risk groups; this technique avoids the requirement of fresh tissue and is easy to carry out in routine clinical practice.

Proliferation and progression of neoplastic cells are known to be closely related to abnormalities in various positive and negative cell-cycle regulators. The p27kip1 protein (p27), a cyclin-dependent kinase (CDK) inhibitor, has an inhibitory effect on the G1-to-S phase transition in the cell cycle through its negative effects on cyclin E/CDK2 and cyclin A/CDK2 [7]. Down-regulation of p27 has been shown to be mediated predominantly through the ubiquitin-dependent proteolytic pathway [8]. Ubiquitin ligase activity in the ubiquitination of p27 is provided by the Skp–Cullin–F-box complex, composed of S-phase kinase-associated protein (Skp) 1, Cullin–1, ring-box 1 (Rbx-1) and the Skp2-specific F-box protein Skp2. Indeed, in human cancers and lymphomas, increased Skp2 expression and reciprocally decreased p27 expression have been demonstrated, indicating an oncogenic
role for Skp2 in carcinogenesis and lymphomagenesis [9–11]. We previously demonstrated that Skp2 expression was a useful factor in determining outcome in patients with DLBCL [12].

Addition of the anti-CD20 mAb rituximab to anthracycline-based chemotherapy, such as the cyclophosphamide, vincristine, doxorubicin and prednisolone (CHOP) regimen, has been shown to improve survival in patients with DLBCL [13]. The clinical applicability of a prognostic factor may depend on a specific therapy, and its usefulness should be reassessed when therapies change [14]. In this study, we used data from 671 patients with DLBCL treated with CHOP with or without rituximab from the Kyushu Lymphoma Study Group to retrospectively analyze whether Skp2 and p27 expression remains a valuable prognostic factor.

### materials and methods

#### patients

All the patients were treated with curative intent with a CHOP-like regimen (n = 425) or with cyclophosphamide, vincristine, doxorubicin and prednisolone plus rituximab (R-CHOP; n = 246) during the period 1995–2005, and clinical follow-ups were carried out up to 31 December 2006 at 22 hospitals in the Kyushu Lymphoma Study Group. Patients were enrolled on the basis of the following criteria: diagnosis of de novo DLBCL, availability of paraffin-embedded tissue obtained at diagnosis before the initiation of therapy and availability of follow-up and outcome data at the treating institutions. Cases of PM DLBCL or primary central nervous system lymphoma were not included in this study. Institutional review board approval was obtained from all the participating institutions. Clinical staging of DLBCL was carried out according to the Ann Arbor classification [15] by physical examination, evaluation of bone marrow specimens and computed tomography of the chest, abdomen and pelvis. The following clinical and laboratory data were available at the time of diagnosis: age, sex, performance status, stage, number of extranodal sites involved, serum lactate dehydrogenase level and the presence or absence of systemic (B) symptoms. On the basis of this information, International Prognostic Index (IPI) scores were determined for 671 patients, and the patients were categorized according to low and low–intermediate risk (low-risk group) and high–intermediate and high risk (high-risk group). None of the patients had a known history of human immunodeficiency virus infection or other forms of immunodeficiency. Histological sections were used to diagnose DLBCL according to the World Health Organization classification of hematopoietic tumors [16] by pathologists at each institute. These sections were reviewed and diagnosis was confirmed at the Department of Pathology, Kurume University School of Medicine.

#### immunohistochemistry

Immunohistochemistry for the Skp2 protein was carried out using a polyclonal antibody against human Skp2 (H-435; Santa Cruz Biotechnology, Santa Cruz, CA) [17]. Staining of ≥40% of lymphoma cells was defined as a high expression of Skp2. Skp2 staining showed a robust and primarily nuclear signal, and the staining intensity did not vary among normal and neoplastic lymphoid cells. Staining and scoring for p27 (clone 57; Transduction Laboratories, Lexington, KY), CD10 (clone 56C6; Novocastra Laboratories, Newcastle upon Tyne, UK), Bcl-6 (clone P1F6; Novocastra Laboratories) and multiple myeloma-1/interferon regulatory factor-4 (MUM1/IRF4, clone MUM1p; Dako, Glostrup, Denmark) were carried out at the Department of Pathology, Kurume University School of Medicine [18], by immunohistochemistry, and GCB and non-GCB DLBCL were classified according to the algorithm of Hans et al. [6]. Cut-off values for high expression were set at ≥20% of cells staining for p27 and ≥30% of cells staining for the CD10, Bcl-6 and MUM1 markers.

#### statistical analysis

Survival curves were estimated using the method of Kaplan–Meier and were compared using the log-rank test. Multivariate regression analysis according to the Cox proportional hazards regression model [18] was carried out with overall survival (OS) or progression-free survival (PFS). The Mann–Whitney U test or chi-square test was used to compare the clinical outcome between patient groups showing high Skp2 expression or low Skp2 expression and high p27 expression or low p27 expression. P < 0.05 was considered significant.

#### results

##### patient characteristics

For the CHOP group, 425 patients aged 23–88 years (median 67.0 years) were investigated. The follow-up period ranged from 7 to 144 months (median 43.2 months). For the R-CHOP group, 246 patients aged 22–90 years (median 68.0 years) were studied. The follow-up period for the R-CHOP group ranged from 7 to 102 months (median 24.0 months). Patient and disease characteristics for both treatment cohorts, including the five clinical parameters comprising the IPI, are listed in Table 1. In the R-CHOP group, OS and PFS were significantly better than in the CHOP group (P = 0.005 for OS, P < 0.001 for PFS). The 3-year OS rate was 63% for the CHOP group and 73% in the R-CHOP group, indicating a survival benefit from the addition of rituximab, consistent with several recent reports [13, 14] (Figure 1A and B). High Skp2 expression was present in 166 of the 425 patients (39%) in the CHOP group and 91 of the 246 patients (37%) in the R-CHOP group. High Skp2 expression was found in both the GCB subtype (115 of 306: 38%) and the non-GCB subtype (142 of 365: 39%). High p27 expression was present in 192 of the 425 patients (45%) in the CHOP group and 154 of the 246 patients (63%) in the R-CHOP group. High p27 expression was found in both the GCB subtype (163 of 306: 53%) and the non-GCB subtype (183 of 365: 50%).

##### outcome analysis of the CHOP group on the basis of Skp2 expression

The relation between Skp2 expression and clinical outcome was examined (Figure 2). The 3-year OS rate was 47% in the high Skp2 group and 73% in the low Skp2 group; the 3-year PFS rate was 37% and 59%, respectively. The OS and PFS times were significantly shorter in patients with high Skp2 expression (both P < 0.001) (Figure 2A and B). We also examined the prognostic impact of Skp2 expression using various cut-off values, and we found that in all cases, Skp2 expression remained a significant predictor of survival. These results indicated that higher Skp2 expression demonstrated worse OS (Figure 2C). In an attempt to exclude the contribution of lymphoma-unrelated deaths, the analysis was repeated in patients aged <74 years (327 of 425 patients, 77%); a similar correlation between Skp2 expression and OS was found (P < 0.001; data not shown). To examine whether the prognostic significance of Skp2 expression was independent of
Table 1. Patient and disease characteristics

<table>
<thead>
<tr>
<th>Study population</th>
<th>CHOP (n = 425)</th>
<th>R-CHOP (n = 426)</th>
<th>CHOP (n = 425)</th>
<th>R-CHOP (n = 426)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHOP</td>
<td>P</td>
<td>R-CHOP</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>p27Low</td>
<td>P</td>
<td>p27Low</td>
</tr>
<tr>
<td></td>
<td>p27High</td>
<td>P</td>
<td>p27High</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>p27Low</td>
<td>P</td>
<td>p27High</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>54.1</td>
<td>53.7</td>
<td>55.4</td>
<td>54.8</td>
</tr>
<tr>
<td>Male (%)</td>
<td>51.2</td>
<td></td>
<td>51.2</td>
<td></td>
</tr>
<tr>
<td>Age (mean)</td>
<td>65.0</td>
<td></td>
<td>65.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>64.3</td>
<td></td>
<td>64.3</td>
<td></td>
</tr>
<tr>
<td>ECOG PS II–IV (%)</td>
<td>20.2</td>
<td></td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25.3</td>
<td></td>
<td>25.3</td>
<td></td>
</tr>
<tr>
<td>LDH &gt; normal (%)</td>
<td>44.7</td>
<td></td>
<td>44.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45.4</td>
<td></td>
<td>45.4</td>
<td></td>
</tr>
<tr>
<td>Clinical stage</td>
<td>17.2</td>
<td></td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.6</td>
<td></td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>IPI &gt; 3 (%)</td>
<td>39.3</td>
<td></td>
<td>37.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40.4</td>
<td></td>
<td>40.6</td>
<td></td>
</tr>
<tr>
<td>Number of extranodal sites (%)</td>
<td>17.2</td>
<td>18.6</td>
<td>17.2</td>
<td>18.6</td>
</tr>
<tr>
<td>p27 expression</td>
<td>50.4</td>
<td></td>
<td>51.8</td>
<td>50.4</td>
</tr>
<tr>
<td>p27 expression</td>
<td>49.2</td>
<td></td>
<td>49.2</td>
<td>49.2</td>
</tr>
</tbody>
</table>
| CHOP, cyclophosphamide, vincristine, doxorubicin and prednisolone; R-CHOP, cyclophosphamide, vincristine, doxorubicin and prednisolone plus rituximab; Skp2, S-phase kinase-associated protein 2; p27, cyclin-dependent kinase-associated protein 27; IPI, International Prognostic Index.

The IPI parameters, a multivariate Cox regression analysis was carried out and we found that the IPI parameters and the expression of Skp2 or p27 were independent predictors of OS (Table 2) and PFS (data not shown). We then specifically examined the indicating power of Skp2 expression in the prognosis for patients with low (n = 258) or high (n = 167) IPI scores. In the subgroup with high IPI scores, patients with high Skp2 expression exhibited significantly shorter OS (P < 0.001) (Figure 2D). An equivalent result was observed in patients with low IPI scores (P < 0.001 for OS) (Figure 2E).

outcome analysis in the R-CHOP group on the basis of Skp2 expression

The relationship between Skp2 expression and clinical outcome was studied in patients treated with R-CHOP. High Skp2 expression correlated with a lower 3-year OS rate compared with low Skp2 expression (50% versus 83%, respectively; P < 0.001). Similarly, the 3-year PFS rate was significantly lower in patients with a high Skp2 expression than in those with low Skp2 expression (45% versus 70%, respectively; P < 0.001). The log-rank test revealed that Skp2 expression correlated significantly with both OS (P < 0.001) and PFS (P < 0.001; Figure 3A and B). We found that Skp2 expression at any cut-off value remained a significant predictor of survival, even in the R-CHOP group. These results indicated that higher Skp2 expression resulted in worse OS (Figure 3C). A multivariate Cox regression analysis revealed that Skp2 or p27 expression was an IPI-independent prognostic marker of OS (Table 2A) and PFS (data not shown) in the R-CHOP group. Analysis of OS in the subgroup with high IPI scores among patients treated with R-CHOP revealed that the high Skp2 group exhibited significantly worse OS (P < 0.001), compared with patients in the low Skp2 expression group (Figure 3D). A similar result was observed in patients with low IPI scores (P = 0.006 for OS; Figure 3E).

survival impact of p27 expression

Patients with low p27 expression showed worse OS and PFS compared with those with high p27 expression in both treatment groups (P < 0.001) (Figure 4A–D).

survival effects of both Skp2 and p27 expression pattern

Survival curves for each subgroup divided by p27 and Skp2 expression were compared (OS in Figure 5A and C; PFS in Figure 5B and D). In the CHOP group (Figure 5A), the subgroup of patients exhibiting high Skp2 and low p27 expression (n = 106) showed the lowest 3-year OS rate (39%), whereas the 3-year OS rate was highest (82%) in the low Skp2 and high p27 expression subgroup (n = 132) (P < 0.001). Similar results were obtained for PFS (Figure 5B) (P < 0.001). In the R-CHOP group, the worst OS and PFS were found in patients with high Skp2 and low p27 expression (Figure 5C and D) compared with other expression patterns of Skp2 and p27 (P < 0.001). Rituximab did not provide any outcome benefit in the high Skp2 and low p27, high Skp2 and low p27 expression groups (Figure 6A–C).
Figure 1. Kaplan–Meier curves of overall survival (OS) (A) and progression-free survival (PFS) (B). In the cyclophosphamide, vincristine, doxorubicin and prednisolone plus rituximab (R-CHOP) group, OS and PFS were significantly better than in the cyclophosphamide, vincristine, doxorubicin and prednisolone (CHOP) group ($P = 0.005$ for OS, $P < 0.001$ for PFS).

Figure 2. Overall survival (OS) and progression-free survival (PFS) of patients with diffuse large B-cell lymphoma (DLBCL) treated with cyclophosphamide, vincristine, doxorubicin and prednisolone (CHOP). Patients were grouped according to their levels of S-phase kinase-associated protein 2 (Skp2) expression. Kaplan–Meier curves of OS (A) and PFS (B) in 425 patients with DLBCL show that Skp2 expression correlated with both OS ($P < 0.001$) and PFS ($P < 0.001$). The degree of Skp2 expression correlated inversely with OS (cut-off values for cell staining: <40%, 40% to <80% and ≥80%) (C). In 167 patients with high International Prognostic Index (IPI) score, those with high Skp2 expression exhibited shorter OS ($P < 0.001$) (D). An equivalent result was observed in patients with low IPI scores ($P < 0.001$ for OS) (E).
Cooperative oncology group performance status; LDH, lactate dehydrogenase.

**Clinical outcome Low Skp2**

- **A. Multivariate analysis for low Skp2 and high Skp2 expression**

**Clinical outcome Low p27**

- **B. Multivariate analysis for low p27 and high p27 expression**

**Clinical outcome CHOP-like therapy**

- **C. Multivariate analysis for CHOP and R-CHOP chemotherapy**

Consistent with our previous reports [12], results of the present study when rituximab is introduced clinically [14, 22, 23].

In addition to results from basic molecular research [10, 11], this clinical study supports a pivotal role for Skp2 in lymphomagenesis. During the cell cycle G1-to-S phase transition, Skp2 degrades p27 [9]. These two proteins are therefore closely associated, and they exhibit a reciprocal expression pattern and prognostic relevance in several cancers [12, 24]. This is clearly shown in Figure 5, where low Skp2/high p27 expression showed good survival compared with high Skp2/low p27 expression. However, 111 of 671 patients (17%)...
showed elevated expression of both p27 and Skp2, and 179 of 671 patients (27%) showed decreased expression of both p27 and Skp2. This implies that DLBCL cells might possess alternative regulatory systems for p27 that are independent of Skp2 ubiquitination. Recent studies have shown that cyclin-dependent kinase subunit (Cks) 1 is also required for the ubiquitination of p27 by bridging Skp2 and its substrate, p27, in vivo and in vitro [25]. Cks1 may also be closely related to p27 expression in some tumors [26]. In addition, systems other than Skp2/Cks1 may be involved in p27 proteolysis. Although p27 is predominantly degraded by Skp2 during the G1–S transition in the nucleus, p27 is also thought to be degraded by the Kip1 ubiquitination-promoting complex system in the cytoplasm during early G1 [27]. This nuclear export of p27 might be regulated by jun-activating domain-binding protein (Jab) 1 [28]. Cks1, Jab1 and other factors, together with Skp2, might play important regulatory roles in p27 degradation.

These additional regulatory proteins might also explain the ambiguous distribution of p27 and Skp2 in clinical samples. Further comprehensive expression studies of these proteins are required to better understand the prognostic relevance of cell cycle-related proteins in patients with lymphoma.

Skp2 has been reported to be an independent prognostic factor in various types of malignancies such as gastric cancers, oral squamous cell cancers and malignant lymphoma [12, 24]. In the present study, Skp2 and p27 protein expression was associated with prognosis in patients with DLBCL treated with CHOP or R-CHOP. In addition, the high Skp2 and low p27 expression group exhibited the most unfavorable prognosis, whereas the low Skp2 and high p27 expression group showed a better prognosis. Multivariate analysis revealed that Skp2 and p27 were independent prognostic factors in patients with DLBCL.

Several target proteins of Skp2-mediated proteolysis have been identified in addition to p27. Mice deficient in Skp2 show
Figure 4. Overall survival (OS) and progression-free survival (PFS) curves of patients with diffuse large B-cell lymphoma (DLBCL) with low p27 and high p27 expression in the cyclophosphamide, vincristine, doxorubicin and prednisolone (CHOP) group (OS: A, PFS: B) and the cyclophosphamide, vincristine, doxorubicin and prednisolone plus rituximab (R-CHOP) group (OS: C, PFS: D). Low expression of p27 was associated with poor prognosis in DLBCL patients in both CHOP and R-CHOP groups.

Figure 5. Overall survival (OS) and progression-free survival (PFS) of patients with diffuse large B-cell lymphoma (DLBCL) on the basis of the pattern of S-phase kinase-associated protein 2 (Skp2) and p27 expression. Kaplan–Meier curves of OS and PFS in 425 patients treated with cyclophosphamide, vincristine, doxorubicin and prednisolone (CHOP) (A and B) and 246 patients treated with cyclophosphamide, vincristine, doxorubicin and prednisolone plus rituximab (R-CHOP) (C and D) are shown according to the expression of Skp2 and p27. The group with high Skp2 and low p27 expression showed the most unfavorable outcome, and the group with low Skp2 and high p27 expression showed significantly better OS and PFS than other expression patterns of Skp2 and p27 ($P < 0.001$).
an intracellular accumulation of p27 and cyclin E [11]. In addition, recent studies have revealed that Skp2 is a transcriptional coactivator of c-Myc, leading to transcriptional activation of target genes and subsequent enhanced cell proliferation and antiapoptosis; Skp2 is also responsible for proteasomal regulation of the Myc protein [29]. The synergistic interaction of c-Myc and Skp2 may enhance the aggressiveness of Skp2-overexpressing lymphoma cells.

Even in the recent rituximab era, the expression pattern of Skp2 and p27 is an important prognostic factor in patients with DLBCL. Therapeutic strategies other than R-CHOP will be needed for patients with DLBCL who exhibit high Skp2 and low p27 expression at the time of diagnosis. Novel therapies that specifically target the cell cycle might be of benefit in subgroup of DLBCL.

**acknowledgements**

This study was carried out in collaboration with the hematologists and pathologists of the Kyushu Lymphoma Study Group. We appreciate all their efforts and expertise. We thank Hironori Koga, Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, for helpful discussions.

**disclosure**

The authors affirm no conflict of interest including financial sources.

**references**


appendix: Kyushu Lymphoma Study Group

Division of Hematology, Department of Medicine, Kurume University School of Medicine: Ritsuko Seki, Ken Tanaka, Takashi Okamura; Department of Pathology, Kurume University School of Medicine: Konomi Takasu, Kennesuke Karube, Koichi Ohshima; Department of Internal Medicine, Matsuyama Red Cross Hospital: Iseung Choi, Tsuyoshi Muta, Tomoaki Fujisaki; Department of Hematology, Kyushu Cancer Center: Naokuni Uike; Department of Internal Medicine, National Hospital Organization Kumamoto Medical Center: Michihiro Hidaka, Toshihiko Murayama, Fumio Kawano; Department of Internal Medicine, Saga Prefectural Hospital, Koseikan: Hisashi Gondo, Fumio Yamasaki; Department of Internal Medicine, Miyazaki Prefectural Hospital: Shigeo Mafuko, Toru Hayashi; Department of Hematology, Hamanomachi Hospital: Tetsuya Eto, Shinichi Aishima; Department of Hematology, Sasebo City General Hospital: Yukiyoshi Moriuchi; Department of Hematology, Iizuka Hospital: Fumihiro Taguchi; Department of Hematology, Harasanshin General Hospital: Tomohiko Kamimura, Shinji Kouno; Division of Clinical Hematology, Kumamoto City Hospital: Hiroyuki Tsuda, Nobuyuki Arima; Shimonskoi City Central Hospital: Ryoosuke Ogawa; Department of Internal Medicine, Gastroenterology and Hematology, Faculty of Medicine, Miyazaki University: Kazuhiro Shimoda, Kiyoshi Yamashita; Department of Internal Medicine, Koga General Hospital: Keiko Suzuki; Department of Internal Medicine, Nippon Telegraph and Telephone Nishinippon Kyushu General Hospital: Hitoshi Suzushima; Molecular Medicine Unit and Hematology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences: Kunihiro Tsukazaki, Masao Tomonaga; Department of Internal Medicine, Kyushu Kosei-nenkin Hospital: Masakazu Higuchi; Department of Hematology, Imamura Bun-in Hospital: Atae Tsunomiyai; Department of Internal Medicine, Saiseikai-Hita Hospital: Masahiro Iwahashi; Department of Medicine and Bioregulatory Science, Graduate School of Medical Science, Kyushu University: Yasunobu Abe; Department of Hematology, Imamura Hon-in Hospital: Toshimasa Kukita, Tadashi Matsumoto; Japanese Red Cross Kumamoto Health Care Center: Minoru Yoshida; First Department of Internal Medicine, Faculty of Medicine, Fukuoka University: Junji Suzumiya, Kazuo Tamura; Department of Hematology, St. Mary’s Hospital: Hiroto Ijoujima, Koichi Higaki, Yutaka Imamura; Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Science: Naoki Harada, Mine Harada; Fukuoka Teishin Hospital: Tsunemitsu Shibuya; Fukuoka Higashi Medical Center: Mika Kuroiwa; Department of Internal Medicine, Kitakyushu Municipal Medical Center: Yuji Ohno; Internal Medicine, Saga University: Eisaburo Sueda; Department of Internal Medicine, Karatsu Red Cross Hospital: Masaharu Miyahara; National Hospital Organization Miyakonojo Hospital: Maeda Koichi; Department of Hematology, Kumamoto University School of Medicine: Fumihiko Matsuno; Department of Hematological and Immunological Medicine, Kagoshima University Hospital: Kimitarou Ouzumi; Division of Endocrinology and Metabolism, Faculty of Medicine, University of Ryukyu: Masato Masuda.