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ORIGINAL ARTICLE

Current and future perspectives on the TARGET system: the registration system for Glivec® established by the JSH

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Abstract Since hematology is a highly specialized field, an individual physician may not have much direct treatment experience with a given disease. Therefore, the Japanese Society of Hematology (JSH) has discussed establishing a registry of hematologic disorders, in order to contribute to improving the quality of treatments and clinical outcomes in this field. As a first step, the Timely and Appropriate Registration System for GLIVEC Therapy (TARGET), a registration system for chronic myeloid leukemia (CML) patients treated with imatinib, was established in October 2003. We present the preliminary results of the first 4 years' experience with this system. CML patients treated with imatinib in Japan were registered through the website and the patient's clinical course, including parameters and events like imatinib dose, blood counts, adverse events, and efficacy were recorded in months 1, 3, 6, 9, and 12 and then every 6 months

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thereafter. By September 2007, 862 patients from 176 hospitals were registered. Follow-up period was 0–54 months, and 127 patients were followed-up for more than 36 months. Based on these cumulative data, present imatinib treatment trends were analyzed and safety and efficacy were compared with international trial data.

Keywords Glivec® · CML · Registration · TARGET · IRIS study · JSH

1 Introduction

In Japan, the molecular-targeting drug imatinib mesylate (Glivec®) was approved and registered in the National Health Insurance Reimbursement List for the treatment of chronic myeloid leukemia (CML) in late 2001. Since then, imatinib has been used as first-line therapy for CML in the chronic phase, achieving excellent outcomes in Japanese patients as has been the case overseas. While imatinib was approved very rapidly by the Japanese Ministry of Health, Labor and Welfare for marketing as a medication to treat CML, the regulatory authorities instructed its manufacturer to perform careful post-marketing surveillance. To ensure that imatinib is used effectively and safely for the treatment of CML, it is necessary to accurately monitor the clinical experience with this drug and distribute the information thus obtained. However, the age-adjusted prevalence of CML in Japan is only 0.76 per 100,000 persons for the general male population and 0.46 for females [1]. Therefore, even hematologists have a limited opportunity to actually treat CML, and it is difficult for them to become expert in the management of this disease. Despite this, the registration of CML patients by their treating physicians and publication of information about the management of CML makes it possible to share experiences among numerous physicians, and thus may allow them to provide better treatment for their patients. Under such circumstances, the JSH founded a Hematological Disease Registration Committee (chaired by Professor Shinji Nakao, Kanazawa University) in 2002 and a "Hematological Disease Database" is also being developed by this society. As part of such activities, CML patients treated with imatinib were prospectively registered from 2003 in order to accumulate efficacy and safety data for this new agent. The registration system for imatinib-treated CML patients is known by the acronym of TARGET, standing for Timely and Appropriate Registration system for Glivec Therapy. Overseas, a large-scale randomized prospective study of imatinib therapy versus conventional interferon-α plus cytarabine (the IRIS Study) is currently underway and the interim results (which are reported almost yearly) have demonstrated the high efficacy of imatinib, but have also revealed the emergence of resistance and various adverse events [2]. The TARGET system is not a clinical trial with clearly defined endpoints like the IRIS study, but instead is a case registration system. However, in the 4 years since its establishment, a large number of patients comparable to that enrolled in the IRIS study have been registered with TARGET and various data have been accumulated. In this article, the operations of the TARGET system and reports on the results obtained by analysis of data derived from this system were presented and discussed.

2 Materials and methods

2.1 Registration and operation of the TARGET system

The senior members of the JSH held repeated discussions with Novartis Pharma (Tokyo, Japan) and CareNet, Inc. (Tokyo, Japan) before setting up the TARGET system on the JSH website in October 2003, using the "ECARESS™" online case registration program of CareNet (Fig. 1). All patients with CML treated with imatinib by the members of the JSH physicians could be consecutively registered in the TARGET system. A treating physician of CML with imatinib can prospectively enter data for each patient receiving imatinib at baseline, after 1, 3, 6, 9, and 12 months of therapy, and every 6 months thereafter with regard to the prescription status of imatinib, treatment outcomes, efficacy, occurrence of adverse events, and safety. Adverse effects of imatinib were graded according to the Common Toxicity Criteria of the National Cancer Institute (NCI-CTC) version 2. These events were evaluated by the same criteria in the IRIS study [2].

Fig. 1 The TARGET system. It opened on the home page of the Japanese Society of Clinical Hematology (JSCH) in October 2003

2.2 Management of the TARGET system

The TARGET system is managed directly by the JSH. While all society members can freely access data from the system, only the members who are actually involved in the management of registered CML patients are permitted to enter information in the database. The clinical data were calculated in real time and shown on the website.

3 Results

3.1 Registration

The number of registered patients has increased since operation of the TARGET system commenced, and the total number of patients reached 862 in September 2007. An average of 13 new patients was registered monthly during the first year after starting up the system. After the financial incentive was added (i.e., the cost of RT-PCR assay for the *BCR-ABL* chimera gene was assigned to Novartis Pharma), the mean monthly number of newly registered patients doubled to 25. The RT-PCR assays were done by SRL, Inc. (Tokyo, Japan) alone until September 2005, but the number of contract laboratories was then increased to three by adding BML, Inc. (Tokyo, Japan) and Mitsubishi Chemical BCL (Tokyo, Japan). Since then, an average of approximately new 20 patients has been registered per month (Fig. 2). The 862 patients registered by September 2007 were mainly from the Kanto region, which includes Tokyo, and the Kansai district. These regions have a very large population and a high number of medical institutions, while the other registered patients were distributed widely around Japan (Fig. 3).

The follow-up status of the 701 patients registered by October 2006 is presented in Fig. 4. At 36 months after the start of TARGET operations, data entry is continuing for 127 patients. However, follow-up ceased for about 200 patients after 3 months, which means that some patients only had initial registration with the TARGET system.

3.2 Clinical results for patients registered in the TARGET system: comparison with the IRIS study and the interim report

3.2.1 IRIS study

In the US and Europe, a phase III randomized controlled trial (the IRIS study) has been comparing imatinib monotherapy ($n = 553$) with conventional standard therapy for CML [i.e., interferon-α plus cytarabine (Ara-C) ($n = 553$)] in previously untreated patients with newly diagnosed CML in the chronic stage [2]. An interim

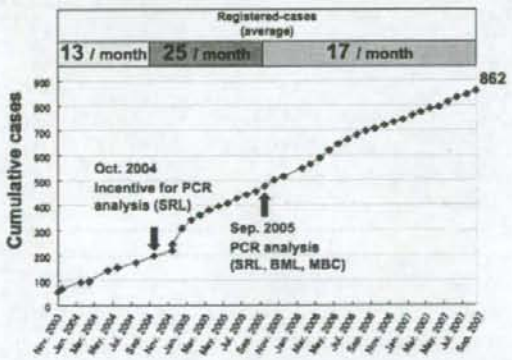

Fig. 2 Numbers of patients registered with the TARGET system from November 2003 to October 2006. The total number of registered patients is 862 as of 30 September 2007. The incentive for RT-PCR analysis of *BCR-ABL* chimeric gene was provided from October 2004. Since then, the mean monthly number of newly registered cases was increased to double

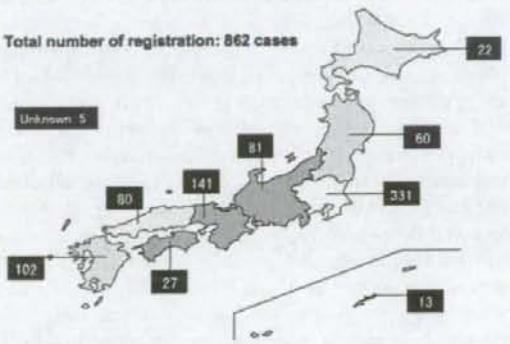

Fig. 3 Distribution of registered patients in Japan. The total 862 patients registered by September 2007 were mainly from the Kanto and Kansai area

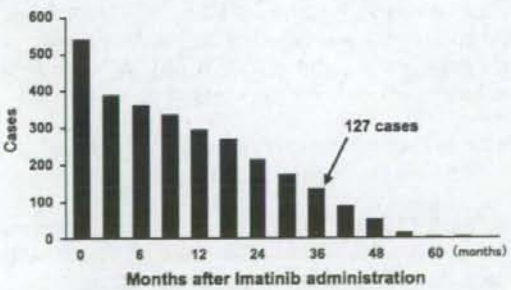

Fig. 4 Follow-up survey of registered patients. The follow-up status of all registered cases were shown, and data entry is continuing for 127 patients at 36 months after the start of the TARGET operations

analysis of this study at 18 months after its commencement showed that imatinib therapy was significantly superior to interferon-based therapy in terms of the cytogenetic complete remission (CCR) rate and prevention of progression to the accelerated phase/blastic phase. Based on these results, imatinib is now positioned as first-line therapy for CML in the chronic stage [2]. In the IRIS study, patients were allowed to cross over to the other therapy if the initially assigned therapy failed to achieve a response, if resistance developed to the assigned therapy, or if intolerance was noted. A total of 318 patients from the interferon-based therapy group transferred to imatinib therapy, with major cytogenetic remission (MCR) being obtained in 55.7% and CCR in 36.9%. In contrast, only 11 patients from the imatinib group transferred to interferon-based therapy and none of them achieved MCR. The recently published 5-year follow-up results showed that CCR was obtained in 69% of patients receiving imatinib after 12 months of treatment and in 87% after 60 months. It was also shown that the overall survival rate of the imatinib group was 89% after 60 months, although 7% of the patients had progressive disease. Thus, imatinib treatment was shown to achieve an excellent outcome over the long term [3]. The IRIS study is still ongoing, and the survival rate has not yet shown a sharp decline in the imatinib group. During this study, BCR-ABL mRNA is measured in the peripheral blood and the presence of minimal residual disease (MRD) is monitored. The results of quantitative PCR have indicated that the BCR-ABL mRNA level decreases by at least 3 logs (1/1,000) after 12 months of treatment in 39% of patients from the imatinib group. In the IRIS patients who showed a reduction of the BCR-ABL mRNA level by at least 3 logs, the progression-free survival rate was 98% even at 42 months after their registration. Based on these results, the target for imatinib therapy has been set as reduction of the BCR-ABL mRNA level by at least 3 logs at an early stage [4]. Since the progression-free rate was significantly higher among patients who obtained CCR or a reduction of BCR-ABL mRNA level by at least 3 logs, the results of 5-year follow-up for the IRIS population also indicate that reduction of BCR-ABL mRNA level by at least 3 logs is an important prognostic factor.

3.2.2 *Efficacy and safety of imatinib in the TARGET population: interim analysis*

Although TARGET is only a case registration system and thus differs in objectives and methodology from the IRIS study that compared imatinib with conventional therapy, we analyzed the data for 378 evaluable patients out of the 701 patients registered with the system up to October 2006 to evaluate the actual use of imatinib and its efficacy and safety for treatment of CML in Japan compared with the interim results of IRIS.

The 378 patients without receiving prior therapy, including interferon registered in the TARGET system were evaluated. They had a median age of 52.2 years and their male/female ratio was 63.8/36.2 (Table 1); therefore, those patients with newly diagnosed and previously untreated CML in the chronic phase were extracted from the TARGET population to match the subjects of the IRIS study, the extracted patients were found to be comparable to the IRIS subjects with regard to their median age, sex ratio, and interval from diagnosis to the start of imatinib therapy (Table 1). Up to 94% of patients were registered as the chronic phase of CML in the TARGET system. Hematology tests performed during imatinib therapy revealed that the median WBC and platelet counts were slightly higher in the TARGET patients, while the median hemoglobin level was similar in the two patient cohorts (Table 1). Thus, although TARGET is only a case registration system, the patient cohort derived from this system was comparable to that of a large-scale controlled study with definitive endpoints, probably because a large number of patients were registered in our system. These results support the validity of analyzing clinical data obtained from the TARGET patient population.

Grade 3 or 4 adverse events detected in the TARGET population were compared with the results of the IRIS study at 18 months. The incidence of superficial edema and

Table 1 Base-line characteristics of patients registered in TARGET system compared with those of IRIS study

	TARGET system ($n = 378$)	IRIS study ($n = 553$)
Age		
Median (year)	52.2	50
Range (year)	16–88	18–70
Sex [no (%)]		
Male	241 (63.8)	341 (61.7)
Female	137 (36.2)	212 (38.3)
Interval since diagnosis (months)		
Median	1.1	2.1
Range	0–60	0–10.4
White cell count ($\times 10^3/\mu l$)		
Median	49.9	17.0
Range	3.2–462	1.6–421.3
Platelet count ($\times 10^3/\mu l$)		
Median	59.2	33.6
Range	6.9–265.2	4.7–295
Hemoglobin (g/dl)		
Median	13	13.0
Range	5.9–20.4	6.9–17.5

hematological adverse events was lower in the TARGET population than those reported for the IRIS cohort (Fig. 5). This might be the difference of patient population in two studies. In the IRIS study, previously untreated patients with newly diagnosed CML in the chronic phase were registered. In contrast, the TARGET cohort included the previously treated patients with interferon-α as well as de novo case of CML. Further investigations with more detailed statistical analyses were needed.

The hematological and cytogenetic responses of all the patients registered with the TARGET system are presented in Fig. 6. After 12 months of imatinib therapy, hematological complete remission (CHR) was obtained in 93.9% of the patients, as well as MCR in 85.6% and CCR in 71.8%. In the IRIS study, the corresponding rates were 96, 85, and 69%, respectively, after 12 months. Thus, the results for the TARGET patient population were similar to those obtained by the IRIS study with respect to the efficacy of imatinib. After 30 months of imatinib therapy, the CHR, MCR, and CCR rates of TARGET patients were 98.3, 96.2, and 91.4%, respectively. However, a cytogenetic study was not done in 23% of the TARGET patients after 12 months of therapy or in 15% after 30 months, thus, these patients were excluded from analysis. This reflects a difference between the controlled IRIS trial, which is performed according to a protocol, and our case registration system. Accordingly, the results for the TARGET patients cannot be compared directly with the IRIS data. Based on analysis of the results obtained by assay of the *BCR-ABL* chimera gene, the IRIS study suggested that a reduction of this gene by at least 3 logs could predict a favorable prognosis [3]. Molecular genetic analysis was also performed in the TARGET patients, and was done in an increasing proportion of them since October 2004, when the cost of quantitative PCR was covered by the JSH up to twice per year for each patient. Unfortunately, quantitative PCR was performed by multiple contract laboratories, and the results are not comparable between each laboratory. Furthermore, there is no standardization regarding which *BCR* and *ABL* genes to use when assessing the expression of the chimera gene. Moreover, data are missing for some TARGET patients, i.e., quantitative PCR was not done at baseline in a relatively large group of patients. These differences also make it difficult to compare results between the TARGET and IRIS cohorts. When we extracted TARGET patients in whom molecular analysis for the *BCR-ABL* chimera gene was carried out using quantitative PCR, we found that the level of expression was reduced by an extent equivalent to 3 logs in about 40% of them after 30 months of imatinib therapy.

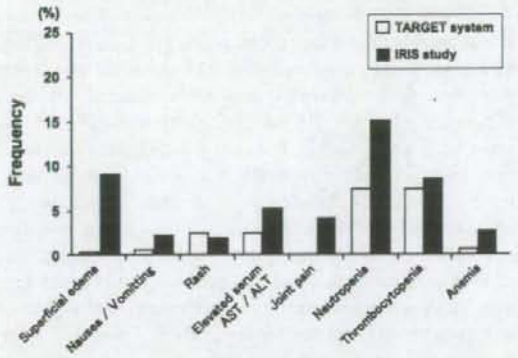

Fig. 5 Adverse events of the TARGET system (*white bar*) compared with these from the IRIS study (*black bar*). Grades 3–4 adverse events including hematological and non-hematological events detected in the registered patients of the TARGET system were compared with the results of the IRIS study at 18 months

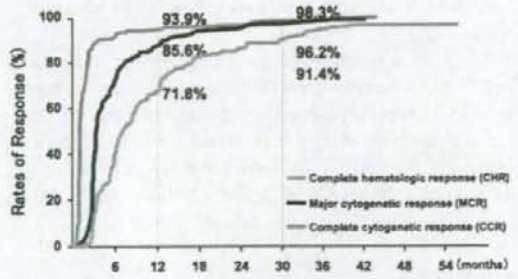

Fig. 6 Kaplan–Meier estimate of the cumulative best response to imatinib therapy for patients registered with the TARGET system. At 12 months after the initiation of imatinib, the estimated rates of having a response were as follows: complete hematologic response (CHR), 93.9%; major cytogenetic response (MCR), 85.6%; and complete cytogenetic response (CCR), 71.8%. At 30 months, the respective rates were 98.3, 96.2, and 91.8%

3.3 Problems with the TARGET system

Since there may be conflicting opinions among participating hematologists, the JSH committee sent a questionnaire to 347 members of the JSH who had registered CML patients in the TARGET system to seek their opinions regarding the following issues: (1) usefulness of the TARGET system for actual clinical management of CML patients, (2) understanding of the concepts behind establishment of the TARGET system, (3) possible improvements to the TARGET system, and (4) the preferred profile/features for epidemiological investigations and registration of treatment outcomes. Replies were obtained from 93 out of 347 physicians (26%).

The TARGET system had a total of 862 registered imatinib-treated patients as of September 2007, and

detailed information has been accumulated in the online database as explained above. However, some operational issues have been proposed by participating hematologists. The most common opinion presented to the TARGET system secretariat is that practising physicians do not have enough time to frequently input data on numerous parameters. Currently, the TARGET system automatically performs analysis of 29 parameters when a patient is registered. Participating physicians also commonly suggested that more parameters useful for the practical management of CML could be assessed.

3.4 Opinions for the TARGET operation

3.4.1 Achievement of the objectives of the TARGET system

The TARGET system was established to disseminate information on the actual clinical management of CML. Regarding whether this objective has been achieved, 77% of the physicians who replied answered "yes."

3.4.2 Clinical usefulness of the TARGET system

Does the TARGET system provide adequate evidence that can "support" decision-making during the management of CML? The most common reply was that it is important for information on actual experience with CML to be made available in real time, while it is also desirable for the results of more detailed analysis to be published. In contrast to clinical trials where the data are only published after a certain interval, it is a great merit of the TARGET system that information obtained during actual treatment of CML can be made available on a real time basis.

3.4.3 Numbers of the registered patients

A total of 862 patients were registered in the TARGET system as of September 2007, and this is thought to account for 10% of all patients treated with imatinib in Japan. This cohort size is thought to be adequate for providing supportive data on the clinical management of CML. Nevertheless, approximately 90% of imatinib-treated CML patients remain outside the TARGET population. Therefore, further efforts are necessary to promote registration with TARGET, while it is also necessary to re-evaluate the number of patients required to obtain adequate clinical data for analysis.

3.4.4 Importance of the registration

Both disease registration to collect information on the onset of diseases and case registration (such as the TARGET system) to collect data on the efficacy and safety of therapy and treatment outcomes should be pursued as society activities. This was the overwhelming opinion of the JSH members who sent replies. In addition, it was considered to be important to accumulate clinical data on Japanese patients with hematological diseases in order to assess disease epidemiology in this country, and accumulated clinical data may also be useful to support the expedited development and approval of new drugs. It was proposed that the JSH should aggressively promote case registration systems like the TARGET system as part of the activities of the society and should ensure that the objectives of such registration systems are widely understood by society members.

3.4.5 Operational issues with the TARGET system

It was revealed that many participating physicians feel that data input is too time-consuming. In fact, initial data input takes from 20 to 30 min per patient. Subsequently, further data are required every 6 months, taking about 10 min for input on each occasion. Because the clinicians who are very busy providing medical care have to input data themselves, it may be necessary to decrease the number of items or the frequency of data entry. Furthermore, in order to continue the use of this system and increase the number of registered patients, it may be necessary to delegate the data input process to staff members other than clinicians and develop appropriate securement measures for such staff.

Quantitative PCR for the *BCR-ABL* chimera gene is available without cost to patients registered in the TARGET system, but no direct incentive is provided for persons dealing with data entry. Some members proposed that an appropriate incentive for data input should also be considered, since it is difficult to actually perform data input with a high level of motivation in the absence of incentives.

Since data on the registered patients are exclusively input by JSH members, the data are considered to be highly reliable. However, analyses should be performed to check for the presence of any bias in the clinical information entered in the TARGET database and to check whether it truly reflects the profile of Japanese patients with CML.

4 Discussion

The primary objective of establishing the TARGET system was to share clinical data on patients with CML receiving imatinib among all hematologists managing CML patients in Japan and thereby improve the quality of medical care for CML in this country. TARGET was also intended to

elevate the profiles of JSH by publishing accumulated data on the treatment of CML. The TARGET database is being compiled to ensure the safety of imatinib and to assess the current status of treatment for CML in Japan, thus supporting the rapid approval process for new drugs with a high clinical need. In particular, there have been rapid advances in the development of novel therapeutic agents for hematological malignancies. For many of these agents, clinical studies have been completed overseas, but they are not yet available in Japan. Thus, the TARGET system that registers patients undergoing imatinib therapy may be utilized for reference when other new drugs are considered for approval in Japan.

The TARGET system is an online database that allows easy access by treating physicians. The system is also designed to increase the accuracy of input data and to maintain security including patient privacy by limiting the persons who can input data to registered physicians who are members of the JSH. The data in the TARGET system therefore have considerable reliability. Moreover, the data are used to make real-time displays that summarize the efficacy and safety of imatinib therapy for Japanese CML patients by producing a total of 29 graphs that are published on the JSH website, therefore, up-to-date clinical information on imatinib is constantly available to hematologists in Japan. Another noteworthy feature is that registered patients are not charged the cost of quantitative PCR assay for the *BCR-ABL* chimera gene up to twice a year, an incentive that was instituted from 1 year after opening the TARGET system. This allows treating physicians to perform more adequate monitoring of the efficacy of imatinib therapy in registered patients, and also helps the patients by reducing their economic burden.

In future of the TARGET system, based on our experience and the results obtained from the questionnaire about the TARGET system, it is considered that case registration systems like TARGET should continue to be part of society activities. The objective of continuing the TARGET system is to confirm the long-term efficacy and safety of imatinib therapy in Japanese patients. Recently, severe heart failure was reported in imatinib-treated patients, and this has also been observed experimentally [5]. Although such an adverse event has not yet been reported among patients registered with the TARGET system, follow-up should be performed to monitor the carcinogenic potential of imatinib and its effect on the immune system and the bones, as well as the occurrence of unknown adverse reactions such as effects on the cardiovascular system. It is also important to clearly define the criteria for analysis of the *BCR-ABL* chimera gene and thus elucidate the efficacy of imatinib in Japanese patients at the molecular level. The emergence of resistance to imatinib therapy has become a concern. In particular, resistance often develops rapidly when imatinib is used to treat CML in the accelerated phase or to treat Ph-positive acute lymphoblastic leukemia (ALL) [6]. The most frequent mechanism of resistance is mutation of the *BCR-ABL* chimera gene [7]. It has been reported that the structure of BCR-ABL protein is altered by amino acid substitution as a result of point mutation, after which imatinib becomes unable to bind to this protein [8]. So far, more than 40 point mutations of the BCR-ABL chimera gene have been detected. Therefore, it is considered important to collect data from patients who discontinue imatinib therapy, patients who develop resistance, and patients with intolerance of imatinib therapy in order to elucidate the outcomes for patients who are not indicated for this therapy and the optimal duration of treatment. Because clinical studies have already been instituted on next-generation tyrosine kinase inhibitors such as dasatinib and nilotinib [9–12], it is of critical importance to establish evidence in Japanese patients supporting a treatment strategy for CML that integrates these new drugs.

In conclusion, the TARGET system is a pioneer registration system for patients receiving treatment of hematological diseases in Japan, and the data obtained are thought to reflect the actual status of CML management in Japan. The results of interim analysis of clinical data from the TARGET system may contribute to improving routine management of CML. To ensure continued operation of the TARGET system, the above-mentioned objectives should be implemented without fail, and more patients should be registered in order to create a comprehensive Japanese database on the treatment of CML. It is also necessary to promote awareness of the value of case registration systems among JSH members as well as modifying the system, and then it becomes more user-friendly. We hope that the results will provide a reference benchmark for improving CML treatment practice in Japan.

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Appendix

The following 428 investigators participated and registered patients for the TARGET system: (Hokkaido) S. Andoh, N. Fuse, Y. Hirata, T. Ishihara, Y. Iuchi, H. Iwasaki, K. Kahata, Y. Kanisawa, M. Kida, H. Kuroda, M. Kurosawa, M. Maemori, N. Masauji, T. Minami, Y. Nagamachi, H. Sakai, M. Shindoh, F. Takahashi, Y. Tsutsumi, S. Yamamoto (Iwate) S. Hoshi, Y. Narigasawa, N. Sakai, A. Satoh, Y. Wano (Miyagi) A. Miura, Y. Saitoh, T. Shishido (Akita) A. Chuhbachi, N. Fujishima, R. Inaba, Y. Kameoka, J. Kuroki, I. Miura, M. Motegi, H. Niitsu, N.

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LETTER TO THE EDITOR

Chronic myeloid leukemia in two patients with gastrointestinal stromal tumor

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Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder. The cytogenetic hallmark of CML is the Philadelphia (Ph) chromosome, which is a shortened chromosome 22 that results from a reciprocal translocation between the long arms of chromosomes 9 and 22. The molecular consequence of this translocation is the fusion of the c-*abl* oncogene from chromosome 9 with sequences from chromosome 22, the breakpoint cluster region (BCR), giving rise to a fused *BCR-ABL* gene. Imatinib mesylate is a potent inhibitor for *ABL* tyrosine kinases, including *BCR-ABL*. It induces cytogenetic and/or hematologic response, improving survival in patients with CML. It has also been shown to inhibit c-*KIT* and the platelet-derived growth factor receptor tyrosine kinases. The *c-KIT*, a transmembrane tyrosine kinase, binds the stem-cell factor and is believed to be essential for the development and maintenance of normal haematopoiesis, mast cells, melanocytes, germ cells, and interstitial cells of Cajal. Gastrointestinal stromal tumors (GIST) are the most common mesenchymal neoplasms of the gastrointestinal tract, which express the c-*KIT* proto-oncogene [1]. Imatinib mesylate targets the surface tyrosine kinase receptor *KIT*. It is approved for treatment of patients with *KIT*-positive unresectable and/or metastatic malignant GIST (Table 1). Here, we report two cases of CML accompanied by GIST. The coincidence of these neoplasms is extremely rare demographically.

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1 Case 1

A 66-year-old male visited our hospital due to leukothrombocytosis on 27 February 2006. He was diagnosed as having CML in the chronic phase with the t(9;22)(q34;q11) chromosomal aberration. A few days later, he consulted a local hospital with a sudden abdominal pain and was diagnosed with acute peritonitis. An emergency laparotomy was performed. A ruptured tumor was found 80 cm from the oral side of the ileum end and was resected completely. Microscopically, spindle-shaped and round epithelioid cells were growing externally from the intestinal muscular propria. Positive immunohistochemical staining for *c-KIT* and CD34 without immunoreactivity to S-100 protein and desmin indicated that it was compatible with GIST. Imatinib mesylate (400 mg/day) was administered to treat CML from the eighth postoperative day. The patient is in complete cytogenetic and complete molecular response for CML without GIST relapse. A complete molecular response was defined as an undetectable *BCR-ABL* transcript level by quantitative polymerase chain reaction, confirmed by a negative nested PCR.

Table 1 Characteristics of two CMLs with gastrointestinal stromal tumor

Characteristics	Case 1		Case 2	
Age/sex	66/male		57/female	
CML				
Peripheral blood				
WBC/μl	66,100		13,600	
Blastoid cell (%)	0		1	
Promyelocyte	0		0	
Myelocyte	1		0	
Metamyelocyte	0		0	
Stab	21		9	
Segmented neutrophil	61		54	
Lymphocyte	13		12	
Monocyte	3		1	
Eosinophil	2		6	
Basophil	0		16	
RBC/μl	430×10^4		443×10^4	
Platelet/μl	79.1×10^4		395.0×10^4	
Chromosome (bone marrow)	46XY, t(9;22)(q34;q11)	19	46XX, t(9;22)(q34;q11)	19
	46XY	1	46XX	1
Major *BCR/ABL*	Positive		Positive	
Stage	Chronic phase		Chronic phase	
Initial treatment of CML	Imatinib mesylate (400 mg/day)		Imatinib mesylate (400 mg/day)	
GIST				
Localization (growing pattern)	Ileum (exophytic)		Stomach (endophytic)	
Tumor size (cm)	$10 \times 7 \times 6$ (ruptured)		$3 \times 2.5 \times 2$	
Immunohistochemistry: KIT/CD34	Positive/positive		Positive/positive	
Risk of aggressive behavior	High		High	
Treatment of GIST	Partial ilectomy		Partial gastrectomy	

2 Case 2

A 57-year-old female was incidentally found to have a submucosal tumor during a regular health check up. Metastatic lesions were not detected, thus the lesion was also completely resected in this case. Pathological examination revealed proliferation of submucosal spindle-shaped cells with elongated cigar-shaped nuclei arranged in fascicles. Immunohistochemical staining confirmed the diagnosis of GIST. Intense positive nuclei on p53 suggested malignancy, although the size was relatively small. The platelet count has generally been increasing since 8 months after operation. She visited our hospital because marked thrombocytosis appeared 12 months after the operation for GIST. The tumor had not recurred at that time. Cytogenetic analysis of neutrophils in peripheral blood showed positivity for the major *BCR-ABL* fusion gene. She was diagnosed as having CML in the chronic phase with the t(9;22)(q34;q11) chromosomal aberration, and started taking imatinib mesylate (400 mg/day). The patient is in complete cytogenetic and complete molecular response without GIST relapse.

In the present report, we demonstrate the supervenience of relatively rare types of tumors: CML and GIST in a single institute. The *BCR/ABL* fusion gene encodes a chimeric protein with strong tyrosine kinase activity, leading to the development of the CML phenotype through processes that are not yet fully understood. On the other hand, the precise mechanism of GIST at the biomolecular level has been clarified recently. Briefly, the gain-of-function mutations of *KIT* in the gastrointestinal tract result in the constitutive activation of *KIT* signaling, which leads to uncontrolled cell proliferation and resistance to apoptosis [2]. There is no relation in etiology between CML and GIST. Therefore, it may be a coincidence that two neoplasms concurrently appeared. Recently, it was reported that patients developed leukemia after GIST, including six cases of acute myeloid leukemia (the interval is from 1.7 to 21 years) and three cases of CML (the interval is from 5 to 20.7 years) [3]. They demonstrated a nonrandom

association between GIST and myeloid leukemia. Many biological data suggest that the initiating lesion in the multistage process leading to cancer might involve destabilization of the genome resulting in elevation of mutation rates [4, 5]. For example, there is strong evidence of such an early genomic destabilization event for colon cancer [5]. Therefore, we suspect that these two cases have some kind of genetic predisposition. Indeed, the first case had a history of early gastric cancer and colon polyps (two years previously). There is also a possibility that the malignancies were provoked by a common undetected mutagen, that is to say, environmental determinants that induce genetic instability. In addition, the disease entity of GIST has been established only very recently, thus we might have missed concurrent GIST in CML patients. Therefore, we should check digestive symptoms carefully in cases with CML.

Imatinib mesylate can induce objective responses and prevent progression of the disease, providing clinical benefit in the majority of GIST patients [6–8]. Although it has not been shown, we expect that imatinib mesylate will prevent the recurrence of GIST as well as CML in our two cases with GIST in remission. Recently and in the near feature, various compounds targeting signal transduction pathways dysregulated in malignancies will be available. Some of these compounds that inhibit several molecules may be potentially effective against a wide range of malignancies.

Accumulation of such cases and further epidemiologic and genetic studies may contribute to clarifying the mechanism of tumorigenesis.

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ORIGINAL ARTICLE: CLINICAL

The expression of anamorsin in diffuse large B cell lymphoma: Possible prognostic biomarker for low IPI patients

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Abstract

Anamorsin is a cell-death-defying factor, which was originally isolated as a molecule that conferred resistance to apoptosis induced by growth factor starvation. In order to evaluate anamorsin expression levels in malignant lymphoma, we immunostained paraffin-embedded sections with anti-anamorsin monoclonal antibodies. About 40% (89/234) of sections from patients with diffuse large B cell lymphoma (DLBCL) showed strong anamorsin expression. Comparing the level of anamorsin expression in DLBCL patients with their clinical features (i.e., overall survival rate, International Prognostic Index (IPI) parameters, and treatment response) revealed no significant correlation between anamorsin expression levels and these clinical features. However, anamorsin expression in DLBCL patients with a low IPI was shown to be an unfavorable biomarker, especially in the patients who received chemotherapy without rituximab. It is suggested that anamorsin might play some roles in the abnormal growth of DLBCL.

Keywords: *Anamorsin, DLBCL, prognosis, IPI*

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma; it is a malignant proliferation of clinically, morphologically and genetically heterogeneous large lymphoid B cells [1]. Recently, the prognostic value of various clinical and biological factors in predicting treatment outcome of DLBCL patients has been discussed [2]. In the early 1990s, a new prognostic model, known as the International Prognostic Index (IPI), was developed based on five clinical, independent predictors [age, stage of disease, extranodal sites, performance status, and serum lactate dehydrogenase (LDH) level] [3]; this index remains the best-validated and most successful tool in predicting survival to date [4]. Furthermore, many prognostic biomarkers including morphologic features, immunophenotypes, cytogenetic abnormalities, expression of apoptosis-related genes, transcription factors, cell-cycle related genes, and gene expression profiling have been examined to predict the outcome of DLBCL patients independently of clinical variables [5,6]. These studies allowed better understanding of the biology of lymphoma and improved the prediction of outcome.

We previously found a cell-death-defying factor named anamorsin (also called CIAPIN-1), which was originally isolated from an interleukin-3 (IL-3)

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independent variant of the mouse IL-3-dependent cell line, Ba/F3, which conferred resistance to factor-deprived apoptosis [7]. Anamorsin is ubiquitously expressed in various organs, including hematopoietic tissues like bone marrow, spleen, and thymus. The expression of anamorsin is also induced by various cytokines including SCF, EPO, and IL-3 through, at least partially, the Ras signaling pathway. Anamorsin-deficient mice die in late gestation. Anamorsin-deficient embryos are anemic; the number of peripheral red blood cells was half that of littermate controls; the size of fetal liver was reduced to one third the size of normal fetal liver. Interestingly, the absolute number of hematopoietic stem cells and immature pro-erythrocytes was almost normal in the fetal liver of anamorsin-deficient embryos, indicating that anamorsin plays a crucial role in hematopoiesis during late and/or terminal stages of differentiation. Although anamorsin has been shown to be an indispensable factor induced by cytokines during hematopoiesis, it remains to be determined if anamorsin participates in the pathogenesis of hematological disorders.

In a preliminary study, we found that anamorsin was overexpressed in a fraction of hematological malignancies, including malignant lymphomas, suggesting that overexpression of anamorsin might play a role in the pathologic features of lymphomas. In order to clarify the biological significance of anamorsin expression in lymphoma, we immunostained paraffin-embedded sections with anti-anamorsin monoclonal antibodies, and analyzed expression levels of anamorsin in a series of patients with DLBCL characterized by a markedly heterogeneous clinical course and response to treatment.

Materials and methods

Patients

Two hundred thirty-four patients with DLBCL, for whom adequate clinical information was available, were selected for the present study. Histologic specimens obtained by biopsy were fixed in 10% formalin, and routinely processed for paraffin embedding. Histologic sections were cut to 4 μm, stained with hematoxylin and eosin, and immunoperoxidase detection procedures were used. All of the histologic sections were reviewed by one of the authors (K.A.), and classified according to the WHO classification. No patient in this study was related with HIV infection.

Immunohistochemical analysis

We generated three kinds of rat monoclonal antibodies against murine anamorsin (KM3048, KM3052, and KM3056) [7]. All antibodies were appropriate for immunoblotting, immunoprecipitation, and immunofluorescence staining. KM3052 and KM3056 reacted with human anamorsin, and could be used for immunostaining of paraffin-embedded sections. Immunohistochemical study on the paraffin sections was carried out using the Envision$^+$ system (DAKO, Carpinteria, CA) as described previously [8]. Briefly, the sections were immersed into Target Retrieval Solution, low pH (DAKO) and heated in a pressure cooker for 40 min for antigen retrieval. KM3052 was usually used as a primary antibody at dilution of 1:800 and Envision$^+$ system-HRP Labeled Polymer (Anti-Mouse) (DAKO) was used as a secondary antibody. The isotype rat IgG_1 (DAKO) was used as a negative control antibody. Sections were counterstained lightly with hematoxylin. KM3052 staining was not detected in anamorsin-deficient embryos, while organs from anamorsin transgenic mice were strongly stained, verifying the specificity of the antibody. Endothelial cells show a constant positive cytoplasmic staining for anamorsin, which was used as internal positive control. Staining intensity in the cytoplasm of the tumor cells was compared with that of endotherial cells.

Germinal center B cell (GCB) type or non-GCB type

In order to evaluate relationship of anamorsin expression with other biological features of the DLBCL patients, we divided the patients into two groups, GCB type or non-GCB type, by the immunophenotyping based on CD10, BCL-6, and/or MUM1 expression pattern proposed by Hans et al. [9] and analyzed the significance of the anamorsin expression in each type. According to the proposal by Hans CP et al., CD10$^+$ cases and CD10$^-$, BCL-6$^+$, MUM-1$^-$ cases were classified as GCB type, while CD10$^-$, BCL-6$^+$, MUM-1$^+$ cases and CD10$^-$, BCL-6$^-$ cases were classified as non-GCB type. Immunohistochemical study was carried out in the same way as anamorsin staining. Anti-CD10 antibody (clone 56C6, Bioscience), anti-BCL-6 antibody (clone PG-B6p, DAKO), and anti-MUM-1 antibody (clone MUM1p, DAKO) were used as a primary antibody and Envision$^+$ system-HRP Labeled Polymer (Anti-Mouse) (DAKO) were used as a secondary antibody.

Statistics

All statistical analyses were performed using JMP software (SAS Institute, Cary, NC). Correlation between anamorsin expression determined by immunohistochemistry, and clinical parameters was

evaluated by using the χ^2 test. Overall survival rates were calculated by the Kaplan-Meier method, and the difference in survival curves was analyzed by the log-rank test; statistically significant differences were denoted by $p < 0.05$.

Results

The clinical features of the 234 patients are summarized in Table I. The Ann Arbor staging system was applied to all 234 patients: stage I in 53 patients, stage II in 65 patients, stage III in 49 patients, and stage IV in 67 patients. Nearly equal numbers of patients occurred in each IPI score: low IPI (number of risk factors, 0–1) in 60 patients, low-intermediate IPI (number of risk factors, 2) in 64 patients, high-intermediate IPI (number of risk factors, 3) in 52 patients, and high IPI (number of risk factors, 4–5) in 58 patients. All patients had received anthracyclin-based chemotherapy, a regimen including doxorubicin, cyclophosphamide, vincristine, and prednisone (CHOP or CHOP-like regimen). The median follow-up period for the 234 patients was 28.4 months. The estimated 5-year survival rate for the 234 patients was 53.5%; 139 (59.4%) of these patients had also received rituximab (anti-CD20 antibody) therapy. The estimated 5-year survival rates for the patients who received combined immunochemotherapy (CHOP or CHOP-like plus rituximab) and the patients who received chemotherapy only were 64.0% and 39.5% respectively (Table II).

Table I. Characteristics of 234 patients with diffuse large B-cell lymphoma.

Characteristics	No. of patients	%	Anamorsin strong expression ($n = 89$)	%
Sex				
Male	132	56.4	54	40.9
Female	102	43.6	35	34.3
Age, years				
<60	75	32.1	33	44.0
≥60	159	67.9	56	35.2
Ann Arbor stage				
I	53	22.6	16	30.2
II	65	27.8	23	35.4
III	49	21.0	18	36.7
IV	67	28.6	32	47.8
Serum LDH level				
Normal or lower	110	47.0	44	40.0
Higher	124	53.0	45	36.3
Performance status				
Ambulatory (0, 1)	171	73.1	64	37.4
Not ambulatory (>1)	63	26.9	25	39.7
Extranodal involvement				
0–1 sites	167	71.4	59	35.3
>1 sites	67	28.6	30	44.8
Size of largest tumor, cm				
≥10	45	19.2	14	31.1
<10	189	80.8	75	39.7
International Prognostic Index				
Low (No. of risk factors, 0–1)	60	25.6	23	38.3
Low-intermediate (2)	64	27.4	18	28.1
High-intermediate (3)	52	22.2	23	44.2
High (4–5)	58	24.8	25	43.1
Treatment response				
CR	156	66.7	57	36.5
PR	51	21.8	17	33.3
SD, PD	27	11.5	15	55.6
Rituximab-containing therapy				
Yes	139	59.4	55	39.6
No	95	40.6	34	35.8

Anamorsin expression

Immunostaining showed that anamorsin was localized in the cytoplasm of lymphoma cells [Figure 1(C)]. Anamorsin staining intensity of tonsils from patients with tonsillitis, or lymph nodes from patients with lymphadenitis showed only weak expression, which was almost the same expression level as seen in the endothelial cells [Figure 1(B)], while the stronger staining intensity of lymphoma cells compared with these organs was considered strong anamorsin expression [Figure 1(C)]. Eighty-nine (38.0%) of 234 patients with DLBCL showed strong anamorsin expression, whereas others showed weak or no detectable expression [Figures 1(B)–1(D)].

Statistical analyses for correlations between anamorsin expression and clinical features

Correlations between anamorsin expression levels and clinical features (sex, age, Ann Arbor stage, serum LDH level, performance status, number of extranodal lesions, size of largest tumor, IPI scores, and treatment response) are summarized in Table I. There was no significant correlation between anamorsin expression levels and these clinical features. However, those patients with stronger anamorsin expression levels responded poorly to treatment ($p = 0.1260$).

Prognostic significance of anamorsin in patients with low IPI scores

Among the 234 patients analyzed in this study, significant differences were seen in overall survival according to the IPI score [Figure 2(A) and Table II], individual prognostic factors (age, Ann

Table II. Univariate analysis for overall survival of patients with diffuse large B-cell lymphoma.

Characteristics	No. of patients (%)	5-year overall survival (%)	*p*
All patients	234	53.5	
Anamorsin expression			
Weak or negative	145 (62.0)	56.2	
Strong	89 (38.0)	49.5	0.5592
Sex			
Male	132 (56.4)	49.8	
Female	102 (43.6)	58.1	0.1629
Age, years			
<60	75 (32.1)	65.1	
≥60	159 (67.9)	47.8	0.0061
Ann Arbor stage			
I	53 (22.6)	76.5	
II	65 (27.8)	67.5	
III	49 (21.0)	37.7	
IV	67 (28.6)	31.3	<0.0001
Serum LDH level			
Normal or lower	110 (47.0)	69.9	
Higher	124 (53.0)	39.8	<0.0001
Performance status			
Ambulatory (0, 1)	171 (73.1)	64.2	
Not ambulatory (>1)	63 (26.9)	22.4	<0.0001
Extranodal involvement			
0–1 sites	167 (71.4)	60.5	
>1 sites	67 (28.6)	34.8	<0.0001
Size of largest tumors, cm			
≥10	45 (19.2)	49.5	
<10	189 (80.8)	54.5	0.7690
International Prognostic Index			
Low (No.of risk factors, 0–1)	60 (25.6)	85.4	
Low-intermediate (2)	64 (27.4)	58.6	
High-intermediate (3)	52 (22.2)	44.1	
High (4–5)	58 (24.8)	20.9	<0.0001
Treatment response			
CR	156 (66.7)	66.9	
PR	51 (21.8)	37.6	
SD, PD	27 (11.5)	4.2	<0.0001
Rituximab-containing therapy			
Yes	139 (59.4)	64.0	
No	95 (40.6)	39.5	<0.0001

Arbor stage, extranodal involvement, performance status, and serum LDH level), and the response status to the first treatment (Table II). The estimated 5-year survival rate for patients with strong anamorsin expression compared to weak or negative expression were 49.5% and 56.2% respectively. There was no significant difference between the overall survival rate of the anamorsin strong-expression group and the weak or negative expression group ($p=0.56$) [Figure 2(B) and Table II]. When the patients were divided into two groups, the patients who received chemotherapy only (CHOP or CHOP-like regimen) and the patients who received combined immunochemotherapy (CHOP or CHOP-like plus rituximab), a significant difference of overall survival rate was shown ($p=0.02$) between the anamorsin strong expression group and the weak or negative expression group in the patients treated without rituximab, while no significant difference was observed in the patients treated with rituximab ($p=0.34$) [Figure 2(C)]. When the patients were divided according to IPI scores, there were no significant correlations between anamorsin expression levels and survival curves in the low-intermediate IPI group ($p=0.84$), the high-intermediate IPI group ($p=0.91$), and the high IPI group ($p=0.18$) (Figure 3). However, in the low IPI group, patients with strong anamorsin expression had a more unfavorable prognosis than those with weak or negative anamorsin expression ($p=0.02$) [Figure 3(A)]. Furthermore, in the low IPI patients treated with chemotherapy, more significant difference of overall survival rate was observed between the patients with strong anamorsin expression and weak or negative anamorsin expression ($p=0.0012$), while a significant difference disappeared in the low IPI patients treated with combined immunochemotherapy ($p=0.69$) [Figure 3(B)]. In the other IPI groups, there was no significant difference of overall survival rate in the patients treated with chemotherapy only as well as in those with combined immunochemotherapy.

Statistical analyses for correlations between anamorsin expression and GCB or non-GCB types

We could classify 176 patients among the 234 patients as GCB type or non-GCB type based on the expression pattern of CD10, BCL-6, and/or MUM-1: 53 patients were GCB type, while 123 patients were non-GCB type. There was no significant correlation between anamorsin expression levels and the immunophenotypes (GCB or non-GCB types) ($p=0.93$). There was also no significant correlation between anamorsin expression levels and survival curves in GCB type ($p=0.10$); however, in non-GCB type, the patients with strong anamorsin expression had a more unfavorable prognosis than those with weak or negative anamorsin expression ($p=0.03$) [Figure 4(A)]. Furthermore, in the non-GCB type patients who received chemotherapy only, more significant difference of overall survival rate was observed between the patients with strong anamorsin expression and weak or negative anamorsin expression ($p=0.001$), while a significant difference disappeared in the non-GCB patients who received combined immunochemotherapy ($p=0.55$) [Figure 4(B)].

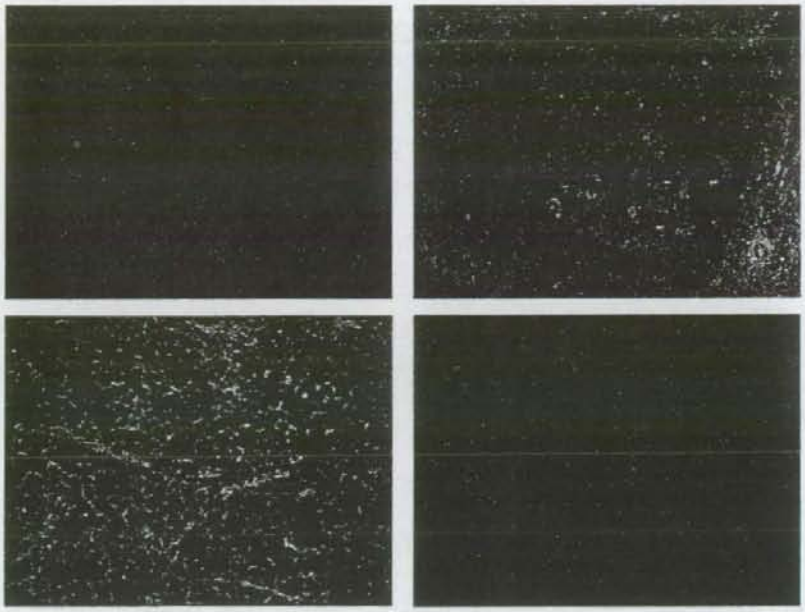

Figure 1. Anamorsin expression in diffuse large B cell lymphoma. A: Immunostained with the isotype control antibody and same sample as C. B–D: Immunostained with anti-anamorsin antibody (KM3052). B: Lymph nodes from patients with lymphadenitis. C: Anamorsin strong expression case. D: Anamorsin negative expression case.

Discussion

Anamorsin was overexpressed in about 40% of patients with DLBCL. In accord with the finding that anamorsin is a cell-death-defying factor, pathological analysis of lymphoid organs from mice with an altered anamorsin gene showed that the spleens of anamorsin-null mice were extremely atrophic [7]. We also generated anamorsin transgenic mice that ubiquitously expressed the anamorsin transgene under control of the cytomegalovirus immediate early enhancer/beta-actin (CAG) promoter. The number of B-lymphocytes in spleens of these mice was increased (unpublished data). From these data, it was suggested that anamorsin might play important roles in normal B lymphopoiesis, and in the pathogenesis of some B cell lymphomas. We were unable to detect any chromosomal translocations related to the anamorsin gene locus (16 q13-q21) in DLBCL patients; therefore, the mechanisms that cause elevated anamorsin expression levels in lymphoma remain to be clarified. A previous report [10] showed the significance of anamorsin in the pathology of cancer. That report showed that anamorsin was overexpressed in multidrug-resistant gastric cancer. Our results showed no statistically significant correlation between anamorsin overexpression and chemotherapy resistance, although there was a tendency that the first chemotherapy failed to produce a response in DLBCL patients with anamorsin overexpression. Consequently, anamorsin might be related to both the pathogenesis and the clinical features of DLBCL.

To understand better the biology of DLBCL and to predict the outcome for DLBCL patients independently of clinical variables, many molecules have been studied to test their worth as prognostic biomarkers. Our study showed the significance of anamorsin as a prognostic biomarker in the low IPI group, suggesting that anamorsin expression might have a powerful impact on the prognosis of low-risk DLBCL patients, who might possess fewer oncogenic alterations. By contrast, it is possible that the effect of anamorsin could be masked with other complicated oncogenic changes in high or middle-risk DLBCL patients [11]. By combining expression profiles of these prognostic biomarkers with clinical parameters, a much more appropriate treatment plan may be achievable for each patient [12,13]. Our results may indicate that intensive chemotherapy or combined immunochemotherapy may be unnecessary for low IPI DLBCL patients who demonstrate weak or undetectable levels of anamorsin expression. Furthermore, by combining two or more biomarkers,

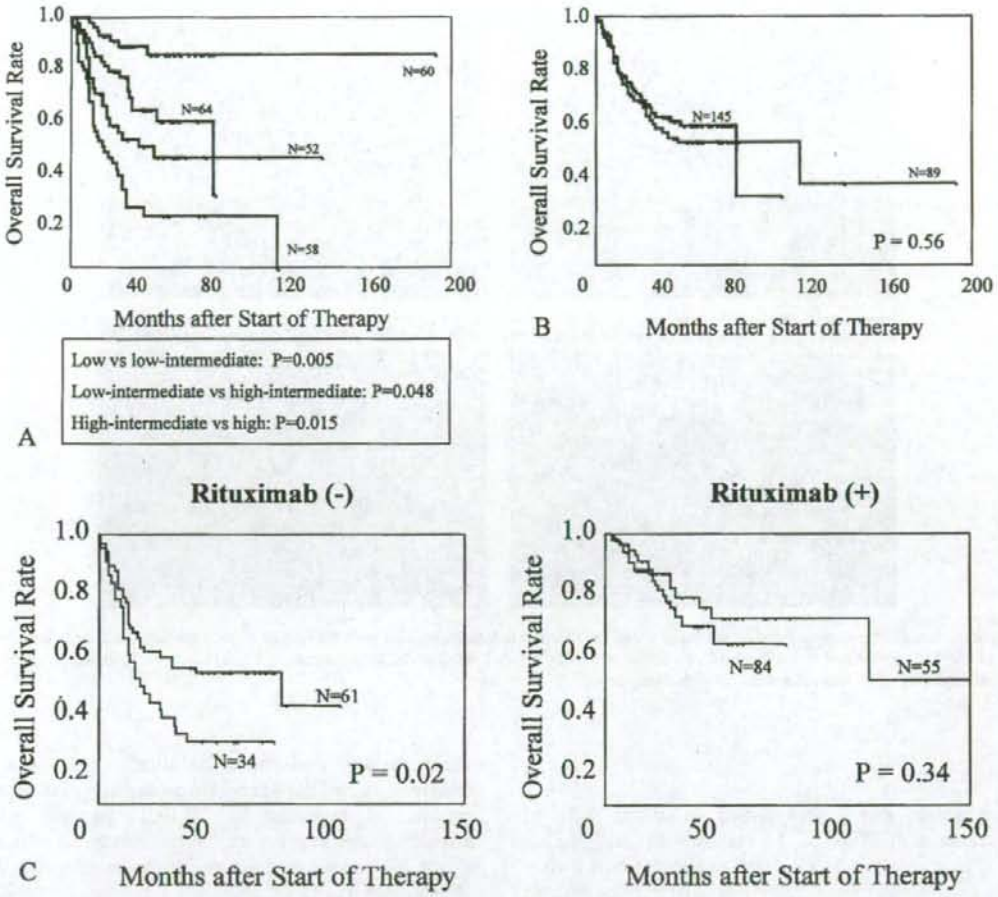

Figure 2. A: Overall survival curves using the Kaplan-Meier method are shown according to the IPI score. — (N = 60): low risk group, = (N = 64): low-intermediate risk group, (N = 52): high-intermediate risk group, and (N = 58): high risk group) Significant differences between these groups are shown by the log-rank test. (Low vs. low-intermediate: $p = 0.005$; low-intermediate vs. high-intermediate: $p = 0.048$; high-intermediate vs. high: $p = 0.015$). B: Overall survival curves using the Kaplan-Meier method are shown according to anamorsin expression levels. (—: weak or negative anamorsin expression group, =: strong anamorsin expression group.) No significant difference is shown by the log-rank test ($p = 0.56$). C: Overall survival curves using the Kaplan-Meier method are shown according to anamorsin expression levels. (—: weak or negative anamorsin expression group, =: strong anamorsin expression group.) A significant difference is shown by the log-rank test in the group receiving chemotherapy only [Rituximab(−)] ($p = 0.02$), while no significant difference is shown in the group receiving combined immunochemotherapy [Rituximab(+)] ($p = 0.34$).

better prediction of the patients' outcome may be possible. Iqbal et al. reported that there was no significant correlation between BCL2 protein expression and overall survival within the GCB subgroup, but BCL2 expression had a significance adverse effect on overall survival within the non-GCB subgroup [14]. As for the anamorsin expression, in non-GCB type, the patients with strong anamorsin expression had a more unfavorable prognosis than those with weak or negative anamorsin expression. From this finding, the non-GCB type patients who demonstrate strong anamorsin expression may require intensive chemotherapy or combined immunochemotherapy. Because this, like many similar studies, was a retrospective analysis, a prospective study should be conducted to assess the true value of anamorsin expression for predicting prognosis and selecting the best treatment option for DLBCL patients.

Recently, a number of molecular target drugs have been developed against cancer, such as monoclonal antibodies to tumor cell surface antigens, and small

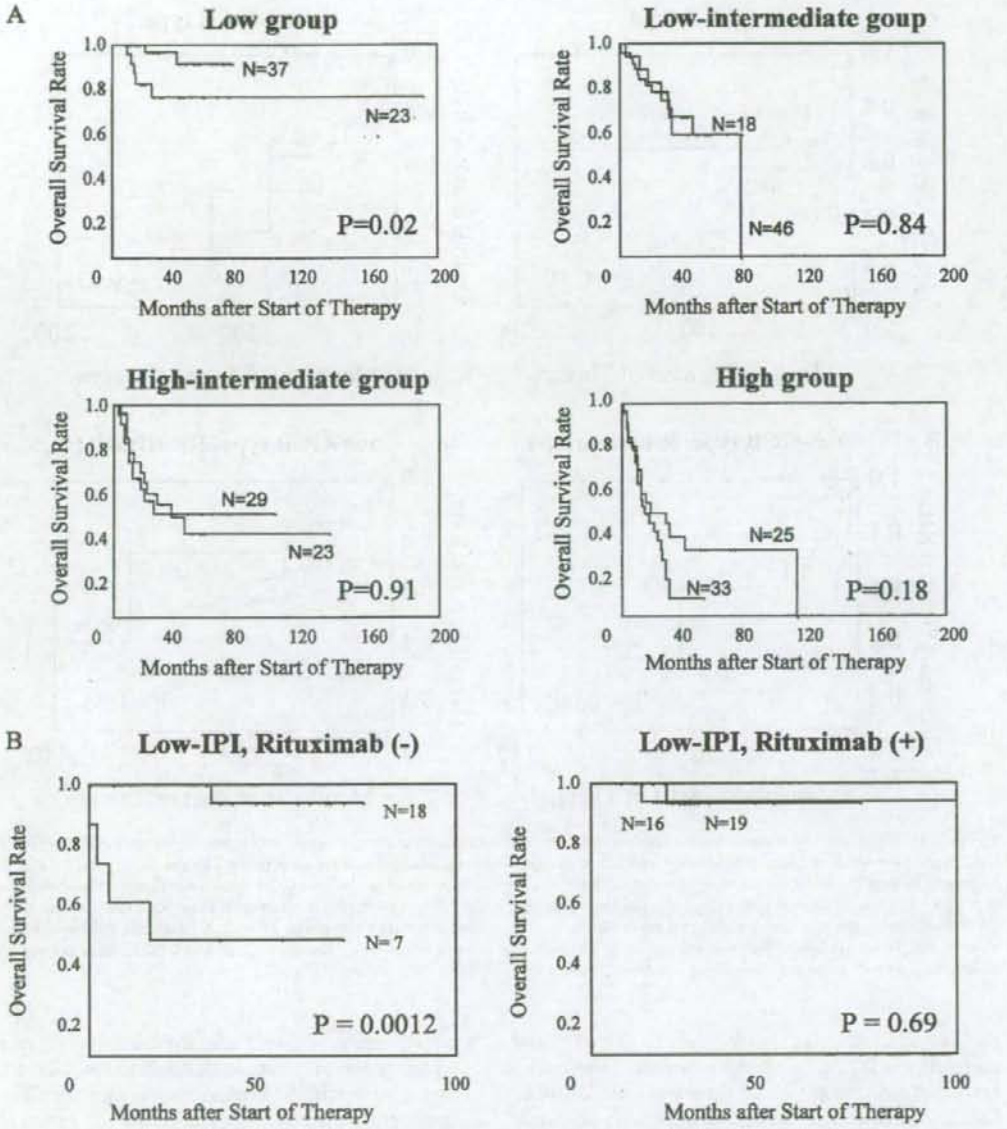

Figure 3. A: Overall survival curves using the Kaplan-Meier method are shown according to anamorsin expression levels within each IPI group. (—: weak or negative anamorsin expression group, ═: strong anamorsin expression group.) In the low IPI group, a significant difference in overall survival rate is shown ($p = 0.02$), while in the other group, no significant difference is shown by the log-rank test. (Low-intermediate IPI group: $p = 0.84$; high-intermediate IPI group: $p = 0.91$; high IPI group: $p = 0.18$). B: Overall survival curves using the Kaplan-Meier method are shown according to anamorsin expression levels within the low IPI group. (—: weak or negative anamorsin expression group, ═: strong anamorsin expression group) A significant difference of overall survival rate is shown by the log-rank test in the group receiving chemotherapy only [Rituximab(−)] ($p = 0.0012$), while no significant difference is shown in the group receiving combined immunochemotherapy [Rituximab(+)] ($p = 0.69$).

compounds like kinase inhibitors [15,16]. Among those drugs, rituximab and imatinib have been very effective [17,18]. In order to develop those novel anti-tumor drugs, further comprehension of the molecular biology of cancer is essential [19]. BCL2 and BCL6 are well-known molecules, both of which