ATL into four clinical subtypes or risk groups, although these factors have not been evaluated simultaneously by a multivariate analysis.[5,19] Of note, these prognostic factors may not have to be applied when considering new therapeutic strategies (eg, antiretroviral therapies).

There are limited data comparing Japanese patients with those in the other countries, and there are no prospective studies addressing this issue.[18,20-22] In a retrospective review of 89 patients predominantly of Caribbean origin, the median age at diagnosis was 50 years, whereas in the Japanese population, it is 57 years.[20] In addition, survival times according to the Shimoyama subclassification in both Caribbean and Japanese populations seem to be comparable (acute: 4 *v* 6 months; lymphomatous: 9 *v* 10 months; chronic: 17 *v* 24 months; and smoldering: 34 months *v* > 5 years, respectively). Although patients of Caribbean origin with less aggressive subtypes fared worse, it is not clear that this is statistically significant.

CLINICAL SUBCLASSIFICATION

Criteria

We recommend following the Shimoyama criteria on ATL clinical subtype classification published in 1991.[19]

Required Evaluation

Involved organ examination: peripheral blood. The diagnosis of ATL requires detection of ATL cells in peripheral blood in patients with acute, chronic, or smoldering type with leukemic manifestations.[4,19] Typical ATL cells have markedly polylobated nuclei with homogeneous and condensed chromatin, small or absent nucleoli, and agranular and basophilic cytoplasm. These so-called flower cells are considered pathognomonic. However, the diversity of recognized ATL cell morphology is considerable.[17,23] Even in patients with extremely unusual morphology, a small percentage of prototype ATL cells have always been seen in blood films, leading to a suspected diagnosis of ATL. This should be confirmed by mature T-cell phenotype, HTLV-1 serology, and monoclonal HTLV-1 provirus in all patients.[17] Five percent or more of abnormal T lymphocytes in peripheral blood confirmed by cytology and immunophenotyping are required to diagnose ATL in patients without histologically proven tumor lesions.[19]

Bone marrow examination. A bone marrow aspiration or biopsy is generally not required to make the diagnosis of ATL. Nevertheless, assessment of the bone marrow may add useful information regarding the normal bone marrow elements before therapy. Furthermore, bone marrow involvement is an independent poor prognostic factor for ATL, similar to that found in peripheral T-cell lymphoma unspecified.[11,24]

Radiologic imaging and endoscopy. Computed tomography (CT) scans of the neck, thorax, abdomen, and pelvis are mandatory to detect sites of nodal and extranodal ATL disease. Upper GI tract endoscopy, with biopsy, should be considered because GI tract involvement is frequent in aggressive ATL.[25] These imaging modalities may detect complicated opportunistic infections including pneumonia, abscess formation, and intestinal infections such as strongyloidiasis and cytomegalovirus.[19] CNS evaluation by radiologic imaging and/or lumbar puncture for cerebral/meningeal ATL involvement or opportunistic infections should be considered for patients in the setting of altered consciousness without hypercalcemia.[26]

Biopsy. When the diagnosis of ATL is not obtained by peripheral blood examination or when a new lesion appears during watchful waiting for indolent ATL, biopsy of suspicious lesion should be performed. Frequently involved tissues include lymph nodes, skin, liver, spleen, lung, GI tract, bone marrow, bone, and CNS.[4-8,11,25,26] As in other types of lymphomas, an excisional biopsy is recommended, instead of core needle biopsy, for lymph nodes. Whenever possible, sufficient sample should be obtained both for histopathologic examination and molecular analyses, including Southern blotting or other (eg, linker-mediated polymerase chain reaction) analysis of HTLV-1 provirus integration.

Tumor marker. Similar to serum LDH reflecting disease bulk/activity, the soluble form of interleukin-2 receptor α-chain is elevated in aggressive ATL patients, indolent ATL patients, and HTLV-1 carriers compared with normal individuals, perhaps with better accuracy than LDH.[27] These serum markers are useful to detect acute transformation of indolent ATL as well as to detect early relapse of ATL after therapy. Serum thymidine kinase levels have also been reported as a promising tumor marker for ATL.[28] However, in the current general practice for the management of ATL patients, only LDH level is required.

Immunophenotype. In most patients, ATL cells exhibit the phenotype of mature $CD4^+$ T cells and express CD2, CD5, CD25, CD45RO, CD29, T-cell receptor $\alpha\beta$, and HLA-DR.[4] Most ATL cells lack CD7 and CD26 and exhibit diminished CD3 expression. Most ATL cells are CD52 positive, but occasionally, patients are negative, and this may correlate with coexpression of CD30. Immunophenotypic analysis of CD3, CD4, CD7, CD8, and CD25 is the minimum requirement for an ATL diagnosis.

Cytogenetics. Karyotypic abnormalities revealed by conventional cytogenetics or comparative genomic hybridization are more common and complex in the acute and lymphoma types compared with the chronic type, with aneuploidy and several hot spots such as 14q and 3p.[16,29] More sensitive array-comparative genomic hybridization revealed that the lymphoma type had significantly more frequent gains at 1q, 2p, 4q, 7p, and 7q and more losses of 10p, 13q, 16q, and 18p, whereas the acute type showed a gain of 3/3p.[30] Currently, outside of clinical trials, cytogenetic analysis is not required.

Molecular biology of HTLV-1. Monoclonal integration of HTLV-1 proviral DNA is found in all cases of ATL as described in the WHO classification.[4] Integration of defective HTLV-1 into ATL cells is observed in approximately one third of ATL patients and is associated with clinical subtypes and prognosis.[31] It is recommended to perform molecular analysis of HTLV-1 integration when possible. Either Southern blotting or polymerase chain reaction for HTLV-1 can be used to identify the presence of viral integration, whereas the latter can be used for quantitative purposes. Seronegativity for HTLV-1 is quite useful to differentiate T-cell lymphomas from ATL, although HTLV-1 is not detected in lymphoma cells other than ATL. Clinically, the diagnosis of ATL is made based on seropositivity for HTLV-1 and histologically and/or cytologically proven peripheral T-cell malignancy, although rare cases of T-cell lymphomas other than ATL developing in HTLV-1 carriers have been observed.[6,8]

Molecular biology of host genome. Mutation or deletion of tumor suppressor genes, such as *p53* or $p15^{INK4B}/p16^{INK4A}$, is observed in approximately half of ATL patients and is associated with clinical subtypes and prognosis.[9,15] These new molecular markers may

help guide therapeutic decisions between conventional chemotherapy, combination of zidovudine (AZT) and interferon alfa (IFN-α), and alloHSCT. In addition to *p53* mutations when considering AZT and IFN-α combination, IRF-4 may be predictive of response.[32]

TREATMENT

Criteria for Treatment Decisions

Treatment decisions should be based on the ATL subclassification and the prognostic factors at onset and response to initial therapy (Table 1). The prognostic factors include clinical factors, such as PS, LDH, age, number of involved lesions, and hypercalcemia, and molecular factors, such as Ki-67 expression, alteration of *p53* or $p15^{INK4B}$/$p16^{INK4A}$, and overexpression of IRF-4.[5,6,8,9,15,19,33-35]

Current Treatment Options

Chemotherapy. The results of a phase III study suggest that, at the expense of higher toxicities, the vincristine, cyclophosphamide, doxorubicin, and prednisone (VCAP); doxorubicin, ranimustine, and prednisone (AMP); and vindesine, etoposide, carboplatin, and prednisone (VECP) regimen is superior to biweekly cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) in newly diagnosed acute, lymphoma, or unfavorable chronic types of ATL.[36] The rate of complete response (CR) was higher in the VCAP-AMP-VECP arm than the biweekly CHOP arm (40% *v* 25%, respectively; $P = .020$). Overall survival (OS) at 3 years was 24% in the VCAP-AMP-VECP arm and 13% in the CHOP arm ($P = .085$). However, the median survival time of 13 months still compares unfavorably to other hematologic malignancies. The superiority of VCAP-AMP-VECP to biweekly CHOP may be explained by the more prolonged, dose-dense schedule of therapy in addition to four more drugs. In addition, agents such as carboplatin and ranimustine that are not affected by multidrug resistance–related genes, which are frequently expressed in ATL cells at onset, were incorporated.[14,36] Intrathecal prophylaxis, which was incorporated in both arms of the phase III study, should be considered for patients with aggressive ATL even in the absence of clinical symptoms because a previous analysis revealed that more than half of relapses at a new site after chemotherapy occurred in the CNS.[37]

IFN-α and AZT. Numerous small phase II studies using AZT and IFN-α have shown responses in ATL patients.[38-42] High-doses of both agents are recommended (6 to 9 million units of IFN-α in combination with daily divided AZT doses of 800 to 1,000 mg/d). However, only patients with wild-type *p53* and low IFN regulatory factor 4 expression seem to exhibit long-term responses to AZT/IFN-α therapy.[32,43,44]

The results of a recent worldwide meta-analysis on the use of AZT/IFN for ATL in 209 patients treated from 1994 to 2006 were presented at the 13th International Conference on Human Retrovirology: HTLV and at the 49th Annual Meeting of the American Society of Hematology.[21,22] One hundred patients received first-line AZT/IFN-α therapy. In these patients, the response rate was 66%, including 43% of patients achieving CR. In patients treated with first-line AZT/IFN-α, the median survival time was 24 months, and the 5-year OS rate was 50%, whereas these values were 7 months and 20%, respectively, in 84 patients who received first-line chemotherapy. The

Table 1. Recommended Strategy for the Treatment of ATL

Smoldering- or favorable chronic-type ATL
Consider inclusion in prospective clinical trials
Symptomatic patients (skin lesions, opportunistic infections, and so on): consider AZT/IFN-α or watch and wait
Asymptomatic patients: consider watch and wait
Unfavorable chronic- or acute-type ATL
Recommend: inclusion in prospective clinical trials
If outside clinical trials, check prognostic factors (including clinical and molecular factors if possible):
Good prognostic factors: consider chemotherapy (VCAP-AMP-VECP evaluated by a randomized phase III trial against biweekly CHOP) or AZT/IFN-α (evaluated by a retrospective worldwide meta-analysis)
Poor prognostic factors: consider chemotherapy followed by conventional or reduced-intensity allogeneic HSCT (evaluated by retrospective or prospective Japanese analyses, respectively)
Poor response to initial therapy with chemotherapy or AZT/IFN-α: consider conventional or reduced-intensity allogeneic HSCT
Lymphoma-type ATL
Recommend: inclusion in prospective clinical trials
If outside clinical trials, consider chemotherapy (VCAP-AMP-VECP)
Check prognostic factors and response to chemotherapy (including clinical and molecular factors if possible):
Favorable prognostic profiles and good response to initial therapy: consider chemotherapy
Unfavorable prognostic profiles or poor response to initial therapy with chemotherapy: consider conventional or reduced-intensity allogeneic HSCT
Options for clinical trials (first line)
Test the effect of up-front allogeneic HSCT
Test promising targeted therapies such as arsenic trioxide + IFN-α, bortezomib + chemotherapy, or antiangiogenic therapy
Consider a phase II global study testing pegylated IFN and AZT
Options for clinical trials (relapse or progressive disease)
Test the effect of promising targeted therapies such as arsenic trioxide and IFN-α, bortezomib, a purine nucleotide phosphorylase inhibitor, histone deacetylase inhibitors, monoclonal antibodies, antiangiogenic therapy, and survivin, β-catenin, syk, and lyn inhibitors, etc.
Consider conventional or reduced-intensity allogeneic HSCT when possible

Abbreviations: ATL, adult T-cell leukemia-lymphoma; AZT, zidovudine; IFN-α, interferon alfa; VCAP-AMP-VECP, vincristine, cyclophosphamide, doxorubicin, and prednisone; doxorubicin, ranimustine, and prednisone; and vindesine, etoposide, carboplatin, and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; HSCT, hematopoietic stem-cell transplantation.

median survival times of patients with acute-type ATL treated with first-line AZT/IFN-α and chemotherapy were 12 and 9 months, respectively. However, achievement of CR with first-line AZT/IFN-α therapy resulted in a prolonged survival time of more than 10 years in 70% of the study population and 75% of the acute-type ATL subgroup. Patients with lymphoma-type ATL did not benefit from AZT/IFN-α therapy; the median survival times of these patients treated with first-line AZT/IFN-α and chemotherapy were 12 and 15 months, respectively. Finally, first-line AZT/IFN-α therapy in chronic- and smoldering-type ATL resulted in 100% OS at a median follow-up time of 5 years. Although the results for AZT/IFN-α in indolent ATL seem to be promising compared with the results seen with watchful waiting until disease progression recently reported from Japan,[45] the possibility of selection bias cannot be ruled out. In conclusion, these results suggest that treatment of ATL using AZT/IFN-α results in high response and CR rates particularly in acute, chronic, and smoldering types of ATL, resulting in prolonged survival in a significant proportion of patients. Although this is a retrospective analysis, the results seem to be promising, and further studies comparing AZT/IFN-α and chemotherapy in acute ATL are warranted.

alloHSCT. alloHSCT is now considered a promising treatment of young patients with aggressive ATL. Despite higher treatment-related mortality in a retrospective multicenter analysis, the estimated 3-year OS rate of 45% is promising, possibly reflecting a graft-versus-ATL effect.[46] A phase I trial of alloHSCT with reduced-intensity conditioning for ATL also revealed promising results. Minimal residual disease after alloHSCT detected by proviral load was much less compared with that after chemotherapy or AZT/IFN-α therapy, suggesting the presence of a graft-versus-ATL effect as well as graft-versus-HTLV-1 activity.[47] It remains uncertain which type of alloHSCT (myeloablative or reduced-intensity conditioning) is most suitable for the treatment of ATL. However, myeloablative alloHSCT, but not reduced-intensity conditioning alloHSCT, might be considered for the treatment of patients with progressive disease (PD) at relapse as well as at onset. Furthermore, selection criteria with respect to response to previous treatments, sources of stem cells, and HTLV-1 viral status of the donor remain to be determined.

Required Pretreatment Evaluation

The diagnosis of ATL is based on HTLV-1 seropositivity and histologically and/or cytologically proven peripheral T-cell malignancy as described in the WHO classification.[4] In uncertain cases, Southern blot hybridization for monoclonal integration of HTLV-1 provirus is useful for the diagnosis, although the sensitivity is to detect the presence of approximately 5% or more monoclonal ATL cells in peripheral-blood mononuclear cells or fresh biopsy.[8]

Traditionally, patients with indolent ATL (ie, the chronic or smoldering type) have been managed similarly to patients with CLL, with a watchful waiting policy until disease progression.[6,8,9] In the consecutive trials for aggressive ATL by Japan Clinical Oncology Group (JCOG)–Lymphoma Study Group, previously untreated patients with aggressive ATL (ie, acute-, lymphoma-, or unfavorable chronic-type ATL) were eligible for participation.[36] Unfavorable chronic-type ATL was defined by at least one of the following three factors: a low serum albumin, high LDH, or high blood urea nitrogen concentration. Unfavorable chronic-type ATL had an unfavorable prognosis similar to acute- or lymphoma-type ATL when treated with chemotherapy.[6] In those trials, other eligibility criteria included no prior chemotherapy, age of 15 to 69 years, and Eastern Cooperative Oncology Group PS of 0 to 3 or 4 as a result of hypercalcemia.[6,36] Eligibility criteria for organ function were also described.[6,36]

Supportive Care

Sulfamethoxazole-trimethoprim and antifungal agents were recommended for the prophylaxis of *Pneumocystis jiroveci* pneumonia and fungal infections, respectively, in the JCOG trials.[6,36] Although cytomegalovirus infection commonly occurs in ATL patients, ganciclovir is not routinely recommended for prophylaxis. In addition, in patients not receiving chemotherapy, antifungal prophylaxis may not be critical. Prophylaxis with anti-*Strongyloides* agents, such as ivermectin or albendazole, should be considered to avoid systemic infection in patients with a history of past and/or present exposure to the parasite in the tropics. Treatment with corticosteroids and proton pump inhibitors may precipitate fulminant *Strongyloides* infestation and warrants testing before these agents are used in endemic areas. It is suggested that *Strongyloides* infection may increase the risk of subsequent development of ATL. Therefore, in HTLV-1 carriers, although not yet demonstrated, prophylaxis of *Strongyloides* may reduce the risk of ATL development.[48-50] Hypercalcemia associated with aggressive ATL should be managed with treatment of the disease, hydration, and bisphosphonate therapy.[6,8]

RESPONSE CRITERIA

The complex presentation of ATL, often with both leukemic and lymphomatous components, makes response assessment difficult; however, response criteria are mandatory to ensure uniform interpretation of clinical trials (Table 2). Most current ATL trials use response criteria proposed by JCOG that have been applied since 1991.[6,36] At the international consensus meetings, a modification of the JCOG criteria was suggested, reflecting the criteria for CLL and NHL that had been published later (Table 2).[51,52] CR was defined as disappearance of all clinical, microscopic, and radiographic evidence of disease. Specific lymph node requirements include that all nodes must have regressed to normal size (≤ 1.5 cm in their greatest transverse diameter) and previously involved nodes that were 1.1 to 1.5 cm must have decreased to ≤ 1.0 cm.[51] Because HTLV-1 carriers frequently have a small percentage of abnormal lymphocytes with polylobated nuclei, so-called flower cells, in peripheral blood, provided that less than 5% of such cells remained, CR was judged to have been attained if the absolute lymphocyte count, including flower cells, was less than 4×10^9/L.[36,52] A designation of unconfirmed CR was adopted to include patients with a ≥ 75% reduction in tumor size but with a residual mass after treatment, as previously reported for NHL.[47] These patients must also have an absolute lymphocyte count, including flower cells, of less than 4×10^9/L. Partial response (PR) was defined as a ≥ 50% reduction in the sum of the products of the greatest diameters of measurable disease without the appearance of new lesions. In addition, PR was required to satisfy a 50% or greater reduction in absolute abnormal lymphocyte counts in peripheral blood. PD in peripheral blood was defined by a ≥ 50% increase from nadir in the count of flower cells and an absolute lymphocyte count, including flower cells, of ≥ 4×10^9/L. PD or relapsed disease in the other lesions was defined as a ≥ 50% increase from nadir in the sum of the products of measurable disease or the appearance of new lesions excluding skin. Stable disease

Table 2. Response Criteria for Adult T-Cell Leukemia-Lymphoma

Response	Definition	Lymph Nodes	Extranodal Masses	Spleen, Liver	Skin	Peripheral Blood	Bone Marrow
Complete remission*	Disappearance of all disease	Normal	Normal	Normal	Normal	Normal†	Normal
Uncertified complete remission*	Stable residual mass in bulky lesion	≥ 75% decrease‡	≥ 75% decrease‡	Normal	Normal	Normal†	Normal
Partial remission*	Regression of disease	≥ 50% decrease‡	≥ 50% decrease‡	No increase	≥ 50% decrease	≥ 50% decrease	Irrelevant
Stable disease*	Failure to attain complete/partial remission and no progressive disease	No change in size	No change in size	No change in size	No change in size	No change	No change
Relapsed disease or progressive disease	New or increased lesions	New or ≥ 50% increase§	New or ≥ 50% increase§	New or ≥ 50% increase	≥ 50% increase	New or ≥ 50% increase‖	Reappearance
Not assessable							

*Require each criterion to be present for a period of at least 4 weeks.
†Provided that < 5% of flower cells remained, complete remission was judged to have been attained if the absolute lymphocyte count, including flower cells, was $< 4 \times 10^9$/L.
‡Calculated by the sum of the products of the greatest diameters of measurable disease.
§Defined by ≥ 50% increase from nadir in the sum of the products of measurable disease.
‖Defined by ≥ 50% increase from nadir in the count of flower cells and an absolute lymphocyte count, including flower cells, of $> 4 \times 10^9$/L.

was defined as failure to attain CR/PR or PD. CR, unconfirmed CR, PR, and stable disease require each criterion for a period of at least 4 weeks.

Recently, revised response criteria were proposed for lymphoma. New guidelines were presented incorporating positron emission tomography (PET), especially for assessment of CR.[53] It is well known and described in the criteria that several kinds of lymphoma, including peripheral T-cell lymphomas, are variably [^{18}F]fluorodeoxyglucose avid.[53] No report described the PET results in response assessment of ATL until now. The usefulness of PET or PET/CT should be evaluated in response assessment of ATL in a prospective study. Meanwhile, PET or PET/CT should be used for evaluation of response when the tumorous lesions are fluorodeoxyglucose avid at diagnosis.

ISSUES FOR FUTURE INVESTIGATIONS IN ATL

Targeted Therapy

Several new agents against ATL are now under investigation. A promising targeted therapy for ATL is the combination of arsenic trioxide and IFN-α, which targets both Tax and the nuclear factor-κB pathway.[54-56] This combination exhibits clinical efficacy in relapsed/refractory ATL patients[57] and is currently being evaluated in untreated patients. Monoclonal antibodies against several molecules expressed on the surface of ATL cells and other lymphoid malignant cells, such as CD25, CD2, CD52, and chemokine receptor 4, have been promising in recent clinical trials. Histone deacetylase inhibitors such as vorinostat (suberoylanilide hydroxamic acid), romidepsin, and panobinostat (LBH589) have also been promising in preclinical and/or clinical studies against T-cell malignancies including ATL. Pralatrexate, a novel antifolate, and forodesine, a purine nucleotide phosphorylase inhibitor, are potential new agents with potent preclinical activity in T-cell malignancies including ATL. Other potential therapies for ATL under investigation include the combination of the proteasome inhibitor bortezomib with high-dose CHOP chemotherapy[58] and antiangiogenic therapy, such as anti–vascular endothelial growth factor monoclonal antibodies[59] or antitransferrin receptor.[60] Microarray analysis has identified survivin, β-catenin, syk, and lyn as potential targets for therapy.[61]

Prevention

Two steps should be considered for the prevention of HTLV-1–associated ATL. The first step is the prevention of HTLV-1 infection. This has been established in some HTLV-1 endemic areas in Japan by screening for HTLV-1 among blood donors and refraining from breast feeding among pregnant women who are carriers. The second step is the prevention of ATL development among HTLV-1 carriers. This has not been established partly because only approximately 5% of HTLV-1 carriers develop the disease in their lifetime and the risk factors remain unknown. Therefore, a cohort study of HTLV-1 carriers (Joint Study of Predisposing Factors for ATL Development) is ongoing nationwide in Japan.

Clinical Trials

Clinical trials have been paramount to the recent advances in ATL treatment, including assessment of chemotherapy, AZT/IFN-α, and alloHSCT, as described earlier. We have proposed a strategy for ATL treatment stratified by subclassification and prognostic factors. However, future clinical trials should be incorporated to ensure that the consensus is continually updated to establish evidence-based practice guidelines.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Phase III study to evaluate the use of high-dose chemotherapy as consolidation of treatment for high-risk postoperative breast cancer: Japan Clinical Oncology Group study, JCOG 9208

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A randomized controlled trial was conducted to evaluate the efficacy of high-dose chemotherapy (HDC) as consolidation of the treatment of high-risk postoperative breast cancer. Patients under 56 years of age with stage I to IIIB breast cancer involving 10 or more axillary lymph nodes were eligible. The primary endpoint was relapse-free survival (RFS). Between May 1993 and March 1999, 97 patients were enrolled, and two patients became ineligible. The median age of the 97 patients was 46 years (range 27–55 years), and 72 (74%) were premenopausal. The median number of involved axillary nodes was 16 (range 10–49). All patients had undergone a radical mastectomy. Major characteristics were well balanced between the treatment arms. Forty-eight patients in the standard-dose (STD) arm received six courses of cyclophosphamide, doxorubicin, and 5-fluorouracil followed by tamoxifen. Forty-nine patients were assigned to undergo HDC with cyclophosphamide and thiotepa after six courses of cyclophosphamide, doxorubicin, and 5-fluorouracil followed by tamoxifen; however, 15 of these patients (31%) did not undergo HDC. HDC was well tolerated without any treatment-related mortality. At a median follow-up of 63 months, the 5-year RFS of 47 eligible patients in the STD arm and 48 eligible patients in the HDC arm was 37% and 52% on an intent-to-treat basis, respectively ($P = 0.17$). Five-year overall survival of all randomized patients was 62% for the STD arm and 63% for the HDC arm ($P = 0.78$). Although the prespecified values of the two arms were not so accurate as to allow detection of the observed difference, no advantage of HDC was observed in terms of RFS or overall survival. (*Cancer Sci* 2008; 99: 145–151)

Preclinical studies have suggested that doses of cytotoxic chemotherapy correlate with the cure of cancer patients.[1] Among several kinds of dose-intensification strategies, high-dose chemotherapy (HDC) with autologous hematopoietic stem cell support has been extensively investigated in clinical oncology. In addition, HDC was shown to produce survival advantages in certain types of malignant neoplasms, including relapsed aggressive non-Hodgkin's lymphoma responding to salvage chemotherapy.[2] and untreated multiple myeloma[3,4] in randomized controlled studies.

Adjuvant chemotherapy has been shown to improve relapse-free survival (RFS) and overall survival (OS) in patients with primary breast cancer[5] and dose-intensification was found to be associated with superior outcomes in some populations.[6] However, the prognosis of patients with extensive axillary lymph node involvement is still poor despite conventional-dose adjuvant chemotherapy. Thus, such patients have been considered to be appropriate candidates for clinical trials of HDC.

Several uncontrolled studies have suggested a survival advantage for HDC in the adjuvant treatment of high-risk primary breast cancer with extensive axillary lymph node involvement.[7–11] At the time of writing, 12 adequately conducted randomized controlled trials comparing HDC with standard-dose (STD) or conventional-dose chemotherapy in high-risk postoperative breast cancer patients have been reported.[12–23] In 10 of them, the advantage of HDC was not shown. However, two of them have shown improved RFS from HDC[18,22] and one study has shown an OS benefit.[22] Thus, its role in the treatment of high-risk primary breast cancer is still inconclusive and deserves further attention.

Based on the promising results of uncontrolled phase II trials of HDC for high-risk primary breast cancer, especially those of the Duke series including patients enrolled into the Cancer and Leukemia Group B (CALGB) study 8782, reported by Peters *et al.*[8] phase I/II studies of cyclophosphamide and thiotepa with autologous bone marrow reinfusion[24,25] and our own earlier feasibility study of HDC of cyclophosphamide and thiotepa with autologous stem cell reinfusion against metastatic breast cancer[26] the Japan Clinical Oncology Group (JCOG)[27] conducted a randomized controlled study to evaluate the efficacy of HDC of cyclophosphamide and thiotepa as consolidation of the treatment for high-risk postoperative breast cancer.

Patients and Methods

Patients. The study was designed for women between 15 and 55 years of age with breast cancer, stage I to IIIB, involving 10 or more axillary nodes, histologically confirmed by level II or further dissection. Eligible patients had to have a performance status rating of 0 or 1 according to the Eastern Cooperative Oncology Group (ECOG) criteria.[28] Exclusion criteria were prior chemotherapy, radiotherapy, and endocrine therapy. Patients

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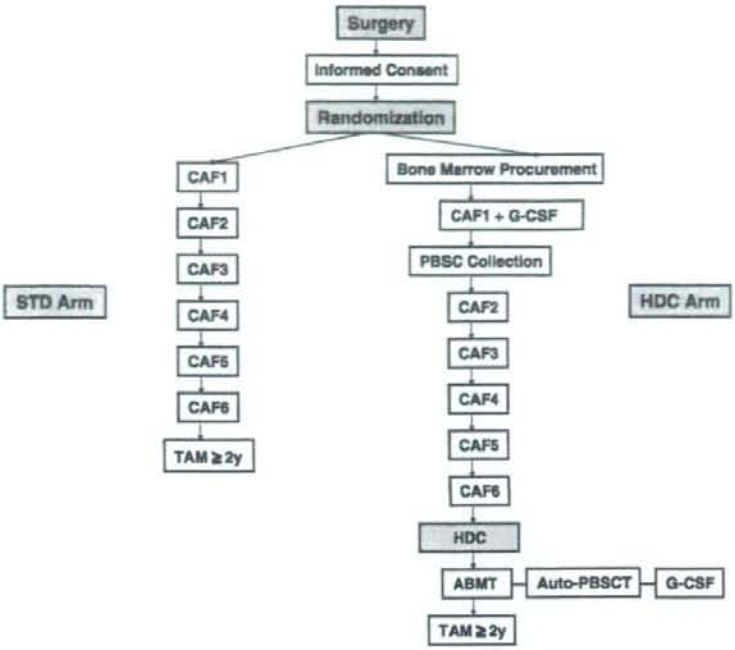

Fig. 1. Trial design of Japan Clinical Oncology Group study, JCOG 9208. ABMT, autologous bone marrow transplantation; CAF, cyclophosphamide, doxorubicin, 5-fluorouracil; G-CSF, granulocyte colony-stimulating factor; HDC, high-dose chemotherapy; PBSC, peripheral blood stem cell; STD, standard-dose; TAM, tamoxifen.

were required to have adequate bone marrow, hepatic, renal, cardiac, and respiratory functions (leukocyte count $\geq 3.5 \times 10^9$/L; hemoglobin ≥10 g/dL; platelet count $\geq 100 \times 10^9$/L; aspartate aminotransferase and alanine aminotransferase ≤4 times the upper normal limit; total bilirubin ≤1.5 times the upper normal limit; blood urea nitrogen and serum creatinine within normal limits; creatinine clearance ≥60 mL/min; no severe cardiac disorder on electrocardiogram; ejection fraction ≥50%; and PaO_2 ≥70 mmHg). Physical examination, chest X-ray, abdominal ultrasound examination, brain computed tomography and a radionuclide bone scan had to be negative for distant metastases. Negative result for bone marrow aspiration or biopsy from the posterior iliac bone was also required.

Patients meeting any one of the following criteria were excluded from the trial: contralateral breast cancer; active concurrent cancer; active peptic ulcer; seropositive for hepatitis B virus surface antigen, hepatitis C virus antibody, or HIV antibody; liver cirrhosis; pulmonary fibrosis or chronic obstructive lung disease; severe psychiatric disorder; diabetes mellitus requiring insulin treatment; uncontrollable hypertension (diastolic pressure ≥110 mmHg); hypercalcemia (serum Ca ≥11 mg/dL); pregnancy or lactation; history of cardiac failure or renal failure; or evidence of concurrent bacterial and fungal infection.

This clinical trial was planned to be conducted at 11 centers belonging to the Autologous Bone Marrow Transplantation Study Group and the Breast Cancer Study Group of JCOG. The JCOG 9208 study protocol and the informed consent document complying with JCOG guidelines and policies were approved by the Clinical Trial Review Committee of JCOG and by the institutional review committee of each participating institution before the start of the study.[27] All patients provided their written or oral consent before the start of the study. Registration involved a telephone call or facsimile from the participating physicians to the JCOG Statistical/Data Center, National Cancer Center, Tokyo, Japan (1991–1997, Statistical Center; 1998–, Data Center).[27] The attending physicians were responsible for submitting periodic data reports on toxicity, relapse, and survival.

Treatment. As shown in Fig. 1, eligible patients were randomly assigned to the STD or HDC arm at the time of enrolment by minimization method to balance the numbers of positive axillary nodes (10–19 or 20–), menopausal status (pre or post) and institution between the arms.

Patients assigned to the STD arm were planned to receive six courses of cyclophosphamide, doxorubicin and 5-fluorouracil (CAF) at 21-day intervals. Each course consisted of intravenous injection with cyclophosphamide 500 mg/m^2, doxorubicin 40 mg/m^2, and 5-fluorouracil 500 mg/m^2. The first course of CAF chemotherapy had to be initiated within 10 weeks after primary surgery.

Patients assigned to the HDC arm underwent bone marrow procurement under general anesthesia before CAF chemotherapy within 9 weeks after primary surgery. Typically, 1 week after primary surgery, they received the first course of CAF together with lenograstim (granulocyte colony-stimulating factor) to collect peripheral blood stem cells (PBSC) as previously described.[29] Lenograstim was given subcutaneously daily from day 8 after CAF chemotherapy until the day of the last leukapheresis. Leukapheresis was carried out once or twice when the leukocyte count increased to greater than 10×10^9/L as described previously.[26] At least 3 weeks after the sixth course of CAF chemotherapy, the patients underwent HDC consisting of cyclophosphamide 2000 mg/m^2/day and thiotepa 200 mg/m^2/day for three consecutive days (days –5 to –3). The doses of cyclophosphamide and thiotepa were determined based on the results of combination phase I/II studies[24,25] and our own feasibility study.[26] Autologous bone marrow and PBSC were thawed and infused on day 0 and 1, respectively. All patients received oral antibiotics, antifungal agents, sulfamethoxazole/trimethoprim and oral acyclovir (200 mg × 5, daily) prophylactically. Irradiated platelet transfusions were given to maintain the platelet count above 20×10^9/L, and irradiated red blood cells were given if necessary. Then 5 μg/kg lenograstim was started on day 2.

Following the above-described therapy, all patients received tamoxifen 20 mg/day for at least 2 years, irrespective of receptor status. Radiation therapy was not planned. All toxicities were

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graded according to the toxicity grading criteria of JCOG,[30] a modified and expanded version of the National Cancer Institute – Common Toxicity Criteria version 1.0.

Baseline evaluation included staging examination (mammography, bone scintigram, brain computed tomography, chest X-ray, abdominal ultrasonography, and bone marrow aspiration/biopsy), complete medical history, physical examination, complete blood cell count, serum chemistry, urinary analysis, tumor marker, and estrogen receptor/progesterone receptor. Restaging evaluation, including chest X-ray, bone scintigram, abdominal ultrasonography, and tumor marker, was conducted every 3–4 months for the first 3 years, and every 6 months for the subsequent 2 years. Central monitoring was carried out every 6 months throughout the study.

Study design and statistical analysis. The primary endpoint was RFS and secondary endpoints were OS and toxicity. RFS was defined as the time from randomization to the first observation of relapse or death due to any cause. OS was defined as the time from randomization to the time of death due to any cause. Survival curves were estimated by the Kaplan–Meier method and compared using the log–rank test.

All eligible patients were analyzed as a data set. To detect a 40% increase in RFS at 5 years of the HDC arm compared with 30% of the STD arm at a significance level of 5% by two-sided log–rank test and a power of 80%, 25 patients are required in each arm. Three years of accrual time and 4 years of follow-up time from the last patient enrolment were assumed initially. As up to 25% of patients in the HDC arm might fail to receive HDC, we estimated a requirement of 100 patients in total (50 patients in each arm) in order to have sufficient statistical power at the beginning of the study.

Patient enrolment into this trial was closed in March 1999, and the actual accrual period was 5.8 years. The follow-up time from the last patient enrolment was amended to 2 years, as approved by the JCOG Data and Safety Monitoring Committee in September 2000.[27] Statistical re-calculation revealed 90% power to detect a 30% increase in RFS at 5 years or 60% power to detect a 20% increase with a significance level of 5% by one-sided log–rank test. No interim analysis was carried out.

Results

Patients. Between May 1993 and March 1999, a total of 97 patients were enrolled from eight institutions. Two patients were ruled ineligible, as one had stage IV disease and the other was enrolled after the start of chemotherapy. Median age was 46 years (range 27–55 years), and 72 patients (74%) were premenopausal. The median number of involved axillary nodes was 16 (range 10–49), and 41 patients (42%) had 20 or more positive axillary nodes. All patients had undergone a radical mastectomy. Forty-eight patients were assigned to receive six courses of CAF (STD arm), and 49 patients were assigned to receive additional HDC with autologous stem cell support (HDC arm). The treatment groups were well balanced in terms of characteristics such as age, menopausal status, performance status, number of positive axillary nodes, stage, and hormone receptor status (Table 1).

Fifteen patients (31%) in the HDC arm did not receive HDC, including seven recurrences during or immediately after CAF therapy, seven refusals and one ineligible patient (Fig. 2). One patient in the HDC arm did not receive high-dose cyclophosphamide on day –3 due to the development of grade 4 arrhythmia (complete atrioventricular block). In addition to the one ineligible, five patients in the STD arm did not complete the planned six courses of CAF therapy, consisting of three recurrences and two refusals. Therefore, of the 97 patients enrolled, 76 (80%) of 95 eligible patients completed the planned treatments.

Major deviations from the protocol were: CAF chemotherapy given despite the presence of grade 2 leukopenia (four patients in the STD arm and nine in the HDC arm); CAF given despite hepatic transaminase elevation >4 times the upper normal limit (one patient in each arm); interval shortening and/or prolongation between the cycles of CAF (four patients in the STD arm and three in the HDC arm); initiation of CAF more than 10 weeks after primary surgery (one HDC patient); and a larger dose (140% of the planned doses) of cyclophosphamide and 5-fluorouracil in the first cycle of CAF (one HDC patient).

Table 1. Characteristics of all randomized patients in the Japan Clinical Oncology Group study, JCOG 9208

Treatment arm		Standard-dose	High-dose
No. of enrolled patients		48	49
Median age in years (range)		47 (27–55)	46 (29–55)
Menopause	Pre/post	34/14	38/11
PS	0/1	41/7	46/3
No. of positive axillary nodes	Median (range)	18 (10–46)	16 (10–49)
	10–	28	28
	20–	20	21
Stage	I	2	2
	IIA	8	12
	IIB	18	16
	IIIA	10	9
	IIIB	10	9
	IV	0	1†
ER	+/–/unknown	29/19/0	25/22/2
PgR	+/–/unknown	25/22/1	22/24/3

†Ineligible. ER, estrogen receptor; No., number; PgR, progesterone receptor; PS, performance status (0 or 1 according to the Eastern Cooperative Oncology Group criteria[28]).

RFS and OS. Seven years after patient recruitment was completed, 52 (54%) of the 97 enrolled patients were alive. Sixty-one (64%) of the 95 eligible patients relapsed or died, 33 (70%) of 47 patients in the STD arm and 28 (58%) of 48 in the HDC arm. Primary analysis was carried out for all 95 eligible patients. At 5 years, RFS of 47 eligible patients in the STD arm and 48 eligible patients in the HDC arm was 37% (95% confidence interval [CI], 23–51%) and 52% (95% CI, 37–66%), respectively (two-sided log–rank, $P = 0.17$) (Fig. 3). Estimated median RFS time was 32 months (95% CI, 23–79 months) for the STD arm and 70 months (95% CI, 36 months–) for the HDC arm. Five-year survival of all randomized patients was 62% (95% CI, 48–76%) for the STD arm and 63% (95% CI, 50–77%) for the HDC arm ($P = 0.78$) (Fig. 4). Estimated median survival time was 87 months for the STD arm (95% CI, 55 months–) and was 110 months for the HDC arm (95% CI, 57 months–).

Toxicity. The HDC treatment was well tolerated, without any treatment-related mortality. All 34 patients receiving HDC actually developed grade 4 leukopenia and grade 4 neutropenia; 27 (79%) developed grade 4 and the other seven grade 3 thrombocytopenia. Hematological status was restored in all patients. Non-hematological toxicities of HDC in 34 patients are shown in Table 2. Three patients developed grade 4 non-hematological toxicities. One developed grade 4 diarrhea on day 4 (9 days after the start of HDC) and recovered 2 days later. Another showed transient grade 4 elevation of hepatic transaminase on day 13 (18 days after the start of HDC). The third patient developed grade 4 arrhythmia (complete atrioventricular block) on day –3 (the third day of HDC), and completely recovered by day 11 (14 days later).

Of 93 patients who actually underwent CAF therapy, seven patients (8%) developed grade 4 neutropenia, but none developed

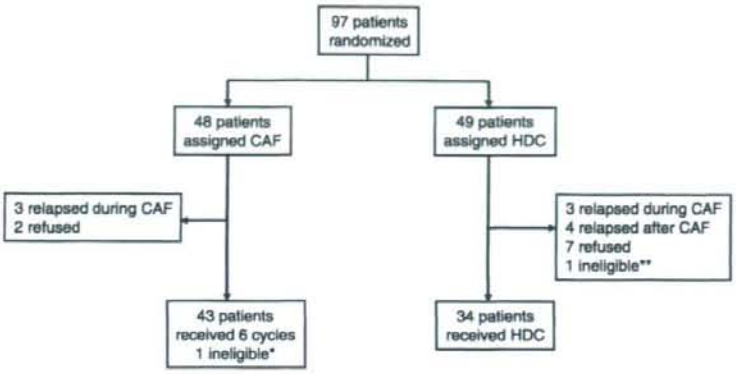

Fig. 2. Trial profile of Japan Clinical Oncology Group study, JCOG 9208. *Registered after the start of cyclophosphamide, doxorubicin, 5-fluorouracil (CAF; violation). **Bone marrow involvement was revealed before the start of CAF.

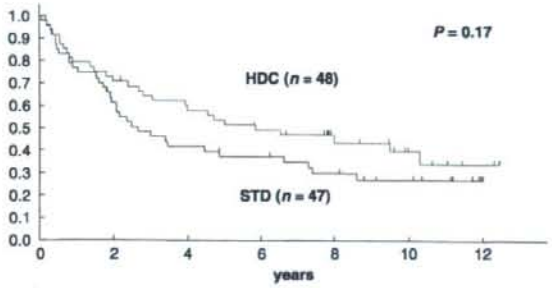

Fig. 3. Relapse-free survival (RFS) of all eligible patients in the Japan Clinical Oncology Group study, JCOG 9208. At 5 years, the intent-to-treat RFS of 47 eligible patients in the standard-dose (STD) arm and 48 eligible patients in the high-dose chemotherapy (HDC) arm was 37% and 52%, respectively (one-sided log–rank, $P = 0.17$). Estimated median RFS time was 36 months for the STD arm and 60 months for the HDC arm.

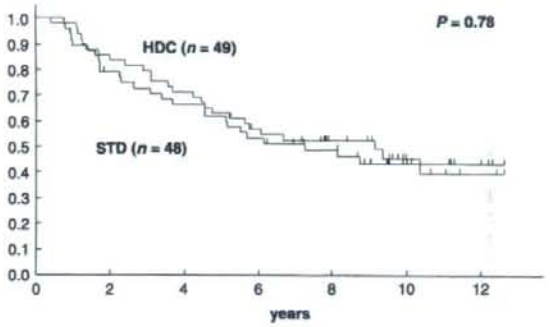

Fig. 4. Overall survival (OS) of all randomized patients in the Japan Clinical Oncology Group study, JCOG 9208. Five-year OS of all randomized patients was 62% for the standard-dose arm and 63% for the high-dose chemotherapy arm (one-sided log–rank, $P = 0.78$).

grade 4 non-hematological toxicities. All the toxicities of CAF therapy were transient.

Discussion

In the present phase III study, we evaluated the efficacy of HDC in high-risk postoperative patients involving 10 or more axillary nodes, using a common CAF regimen as an induction therapy, and HDC as a consolidation after CAF therapy. So far, 13 randomized controlled studies to evaluate the use of HDC in high-risk primary breast cancer have been reported[12–23] including the first report of our study.[31] In the present report, we have updated the analysis of the study, now with a median follow-up of 63 months. However, our study was unable to show any advantage of HDC in terms of RFS or OS.

In our first report, the 4-year RFS of the STD arm was 43% and that of the HDC arm was 61%, showing a trend favoring the latter, although there was no statistical significance between the two arms ($P = 0.12$).[31] In this analysis, the 5-year RFS of the STD and HDC arms was 37% and 52%, respectively, again

Table 2. Non-hematological toxicities of high-dose chemotherapy in 34 patients in the Japan Clinical Oncology Group study, JCOG 9208

Toxicity	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)	Any grade (%)
Nausea/vomiting	3 (9)	9 (26)	22 (65)	0	34 (100)
Diarrhea	10 (29)	11 (32)	9 (26)	1 (3)	31 (91)
Mucositis	16 (47)	3 (9)	5 (15)	0 (0)	24 (71)
Arrhythmia	3 (9)	1 (3)	1 (3)	0 (0)	6 (18)
Infection	9 (26)	9 (26)	2 (6)	1 (3)	20 (59)
Bilirubin	0	4 (12)	1 (3)	0 (0)	5 (15)
AST	15 (44)	12 (35)	5 (15)	0 (0)	32 (94)
ALT	10 (29)	13 (38)	7 (21)	1 (3)	31 (91)

No therapy-related death was observed during high-dose chemotherapy. All toxicities were graded according to the toxicity grading criteria of JCOG,[30] a modified and expanded version of the National Cancer Institute – Common Toxicity Criteria version 1.0. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

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without statistical significance. When we designed this randomized study in 1992, we anticipated a 5-year RFS of 30% for the STD arm, based on the results of consecutive clinical trials conducted by CALGB (CALGB 7581 and CALGB 8082)[8,32,33] and the historical series in the National Cancer Center Hospital in Japan; the 5-year RFS and 10-year RFS of patients involving 10 or more axillary nodes were 30% and 19%, respectively (unpublished data). However, we expected a 5-year RFS of 70% for the HDC arm, based on the results of the phase II study by the Duke group.[8] When we took a closer look at these results, and in particular the selection biases in phase II studies, it seemed likely that the expected difference in RFS between the two arms was too large. As the present study was small and did not have sufficient statistical power to detect small differences (90% power to detect 30% increase in RFS at 5 years or 60% power to detect 20% increase with a significance level of 5% by one-sided test), there remains a possibility that a smaller advantage for the HDC was missed. However, the absence of a trend favoring the HDC arm in OS ($P = 0.75$) suggests that the survival advantage for the HDC would be minimal even if it exists.

The 4-year RFS of 61% for the HDC arm was similar to that of the collected data of the Autologous Blood and Marrow Transplant Registry (ABMTR).[34] However, the RFS data for the HDC arm in the present study was inferior to that of the Duke series (63% of 5-year RFS in the present study *versus* 71% in 5-year event-free survival [EFS] in the Duke series).[16] In the Duke series, only 10% of patients had 20 or more axillary node metastases (median, 14), whereas it was 41% of patients in the present study (median, 16). The higher RFS in the Duke series could be explained partly because they contained more patients with lower risk than the present study. Another possible explanation is that cyclophosphamide and thiotepa of the HDC regimen used in the present study might be less active than cyclophosphamide, carmustine, and cisplatin used in the Duke series.[7,8] The cyclophosphamide and thiotepa regimen was most common in HDC for stage II or III or inflammatory breast cancer, followed by the cyclophosphamide, thiotepa, and carboplatin regimen, according to analysis of ABMTR.[34] Although these two regimens have never been directly compared in a randomized fashion, the analysis of 3451 metastatic breast cancer patients in ABMTR suggested that the HDC regimen did not affect prognosis.[34] Furthermore, two other studies recruiting patients with 10 or more positive axillary nodes[19,20] showed a 6-year RFS of 48% and a 4-year RFS of 52% for the HDC arms, respectively, similar to our results.

In contrast to the RFS results in the HDC arm, the 5-year RFS of 37% for the STD arm was higher than initially anticipated. According to the abstract for the annual meeting of ASCO in 1992 by Peters *et al.*[8] 3-year EFS of the historical control series from CALGB using adjustment for duration of follow-up and selected for age less than 56 years, involvement of 10 or more axillary nodes, and freedom from failure of at least 5 months was 30% in CALGB 8082 and 38% in CALGB 7581.[8,32,33] In an intergroup phase III study, 6-year RFS of 257 patients with 10 or more positive nodes in the conventional-dose arm was 46%[19] and in a German study, 4-year RFS in the conventional-dose arm was 42%.[20] Thus, it is unlikely that RFS in the conventional-dose arm was too high in the present study. As Peto commented on the trend towards a sizeable reduction in breast cancer mortality during the last decade, small improvements might add up to a large beneficial effect[35] in addition to patient selection[36,37] and stage migration.[38]

In the present study, all patients received tamoxifen 20 mg/day for at least 2 years, irrespective of receptor status. In the German study[20] tamoxifen was not planned in the initial protocol, although it was amended.to prescribe tamoxifen for patients with positive hormone-receptor status simultaneously in the HDC and STD arms. According to the Dutch study protocol[15] all patients originally received tamoxifen (40 mg/day) for 2 years. Because of the increasing evidence for treatment with tamoxifen in hormone receptor-positive patients, the protocol was amended and only patients with hormone receptor-positive cancer continued to receive tamoxifen for an additional 3 years. On the contrary, in the ECOG study[19] tamoxifen (20 mg/day) was to be given for 5 years to hormone receptor-positive patients in line with current recommendations. Furthermore, in the present study, adjuvant radiotherapy was originally prohibited, as regional radiotherapy had not been established when the protocol was designed. In the German study[20] as well as the Dutch study[15] it was not initially specified. In contrast, 50 Gy of regional radiotherapy was to be given in the ECOG study.[19] Thus, even in terms of tamoxifen treatment and regional radiotherapy after chemotherapy, protocols in the trials were varied. The results from the single trials and the meta-analysis were inconclusive. HDC should be further investigated in the context of contemporary therapies such as taxanes, dose-dense therapy, hormonal therapy, and radiotherapy.

Of 49 patients assigned to the HDC arm, 15 patients (31%) did not undergo the HDC, which was more than expected (up to 25%). Seven had relapsed before HDC, and seven refused it. When we compared the 69% (34/49) of patients in the HDC arm actually receiving HDC with the results of large studies (96% [264/274] in a Scandinavian study,[14] 90% [397/442] in a Dutch national phase III study,[15] 84% [214/254] in the ECOG study,[19] and 82% [123/150] in the German study[20]), fewer patients could complete HDC in the present study. In the US intergroup trial,[16] randomization was carried out after completion of the induction chemotherapy. This might have been a better option for the present trial.

In the present study, the effectiveness of HDC as consolidation was not confirmed in patients with high-risk postoperative breast cancer involving 10 or more axillary nodes. In the PEGASE 01 trial ($n = 314$) enrolling patients with eight or more positive axillary nodes, 3-year RFS was 71% and 55% ($P = 0.002$) for the HDC and STD arms, respectively.[18] Recently, Nitz *et al.* published the most successful results of HDC in the West German Study Group study.[22] In that study, tandem HDC was compared with dose-dense chemotherapy in 403 patients with at least nine positive nodes (mean, 17.6). Patients in the HDC arm received two cycles of standard-dose EC (epirubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2) at 2-week intervals followed by two cycles of HDC with epirubicin 90 mg/m^2, cyclophosphamide 3000 mg/m^2, and thiotepa 400 mg/m^2 every 21 days with autologous hematopoietic stem cell support. Patients in the control arm received dose-dense chemotherapy with four cycles of standard-dose EC followed by three cycles of cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2, and 5-fluorouracil 600 mg/m^2 at 2-week intervals. With a median follow-up time of 48.6 months, 4-year EFS was 60% in the HDC arm and 44% in the control arm ($P = 0.00069$). The 4-year OS rates were 75% and 70% ($P = 0.02$), respectively. Although an early and rapidly cycled tandem HDC might be a promising approach to be prospectively examined, the efficacy of HDC in the treatment of high-risk primary breast cancer nonetheless remains inconclusive.

Retrospective subgroup analyses to find subsets with more benefit from HDC have been reported, but because of the limited sample size this could not be carried out in the present study. In the Dutch study, patients with HER2-negative disease benefited from HDC with a hazard ratio (HR) of 0.68 for RFS ($P = 0.002$) and 0.72 for OS ($P = 0.02$).[39] In the West German Study Group trial, retrospective subgroup analyses for triple negative patients showed that tandem HDC did significantly better than the control arm in terms of RFS (HR = 0.31) and OS (HR = 0.35, $P = 0.011$).[40]

In the present study, no treatment-related death occurred in either treatment arm. The ABMTR database reported that 3% of

patients treated with HDC died within 100 days after transplantation in stage II or III or inflammatory breast cancer.[34] Peters *et al.* reported a treatment-related mortality of 12% in the Duke series[7] and 7% in the HDC arm in the US intergroup trial.[16] Although the present trial was the first multi-institutional study using HDC for primary breast cancer patients in Japan, HDC could be safely used by the JCOG members.

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Diffuse large B-cell lymphoma after transformation from low-grade follicular lymphoma: morphological, immunohistochemical, and FISH analyses

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Follicular lymphoma (FL) is one of the most common subtypes of non-Hodgkin lymphoma and frequently transforms to diffuse large B-cell lymphoma (DLBCL). To clarify some aspects of the natural history of FL, we retrospectively examined 43 consecutive patients who had DLBCL with pre- or coexisting FL grade 1 or 2. The patients comprised 22 men and 21 women with a median age of 53 years. Most of the patients (34/43) showed advanced-stage (III or IV) disease initially. We examined both FL and DLBCL components morphologically, immunohistochemically, and by interface fluorescence *in situ* hybridization (FISH: IGH/BCL2 fusion, *BCL6 translocation*) analysis. Most of the DLBCLs were classified as the centroblastic subtype, with two exceptions of the anaplastic subtype. Immunohistochemical analysis of both the FL and DLBCL components revealed the following respective positivity rates: CD20 100%/100%, CD10 86%/66%, Bcl-2 96%/91%, Bcl-6 84%/88%, MUM1 16%/34%, CD30 0%/20%, CD138 0%/0%, and CD5 0%/3%. Loss of CD10 (*6/36, 17%*) and gain of MUM1 (*7/28, 25%*) and CD30 (*5/21, 24%*) through transformation were not infrequent. High positivity rates for Bcl-2 and Bcl-6 were maintained throughout transformation. Among the DLBCLs, 84% were classified as the germinal center B-cell phenotype (GCB) and 16% as non-GCB in accordance with the criteria of Hans *et al.* IGH/BCL2 fusion was detected by FISH in 89% of FLs and 82% of DLBCLs. BCL6 translocation was detected in 1/6 (17%) DLBCLs without IGH/BCL2 fusion. Thus, although the morphological features and FISH results for DLBCL were consistent with transformed FL, the immunophenotype showed wide heterogeneity. (*Cancer Sci* 2008; 99: 1760–1768)

Follicular lymphoma (FL) is one of the most common subtypes of non-Hodgkin lymphoma in the Western world, accounting for 22% of all cases worldwide.[1] FL occurs over a broad range of ages, and most cases are manifested initially in lymph nodes.

The risk of FL transformation has been reported as being approximately 20% at 8 years.[2,3] Transformation to DLBCL is observed frequently, with cells most commonly resembling centroblasts,[4] but occasionally resembling anaplastic large cells with CD30 expression.[5] Rare cases have transformed to Burkitt or Burkitt-like lymphoma,[6] or precursor B-lymphoblastic lymphoma/acute lymphoid leukemia.[7] Moreover, composite FL and Hodgkin lymphoma have been suggested to represent two morphologic manifestations of the same tumor clones.[8,9]

In recent years, several analyses of genetic alterations that appear to affect the risk for FL transformation have been reported, including c-MYC translocation,[6] p53 mutation,[10,11] deletions of the tumor suppressor genes p15 and p16,[12,13] and chromosomal 6q23-26 and 17q aberrations.[14] However, there have been few immunohistochemical analyses of transformed FL. FL is positive for the pan-B-cell marker CD20, and frequently positive for CD10, Bcl-2 and Bcl-6, but is usually negative for the postgerminal center B-cell or plasma cell markers CD30, MUM1 and CD138, and CD5. Because only one study has demonstrated gain of CD30 expression through FL transformation,[5] we considered that more analyses were needed to clarify the immunophenotypic changes occurring during the transformation of FL to DLBCL.

Since 2000 DLBCL has been subdivided into GCB and non-GCB (including the activated B-cell phenotype [ABC] and type 3 phenotype) using the cDNA microarray technique.[15,16] The GCB group shows better outcomes and includes cases with translocation (14;18)(q32;q21). For clinical practice, Hans *et al.* showed that a panel of immunohistochemical markers comprising CD10, Bcl-6, and MUM1 could be used on paraffin-embedded tissues to separate DLBCL into tumors with a GCB or non-GCB phenotype.[17] Davies *et al.* examined 35 cases of transformed FL, and found that 89% of them had a GCB phenotype and 9% had a non-GCB phenotype.[18]

The translocation (14;18)(q32;q21) is present in 80–100% of FLs in Western countries,[19,20] whereas in South-East Asia, including Japan, the incidence of translocation is considerably lower: about 60%.[21] It is unclear whether FL with t(14;18) frequently transforms to DLBCL.

The aim of this study was to clarify the natural history of FL, mainly in view of immunophenotypic changes through transformations. We evaluated low-grade FLs and their transformant DLBCLs using morphological, immunhistochemical, and FISH analyses to delineate the heterogeneity of DLBCL after transformation from low-grade FL.

Materials and Methods

Patients. The criteria used for identification of transformed FL were those reported for aggressive B-cell lymphomas in a review based on the workshop of the XIth Meeting of the European Association for Haematopathology.[22] Briefly, it was considered that the term 'transformation' should be used only when there was morphological evidence of simultaneous or prior low-grade FL. Therefore, in the present study, we chose DLBCL with simultaneous or prior low-grade (grade 1 or 2) FL. We retrospectively studied 43 consecutive patients with DLBCL with pre- (20 cases) or coexisting (23 cases) FL grade 1 or 2 treated at the National Cancer Center Hospital, Tokyo, Japan, between 1997 and 2005. The total number of DLBCL specimens was 47 (1–3 per case), and the total number of specimens of low-grade FL was 53 (1–5 per case). A total of 400 FLs and 653 DLBCLs were registered during the same

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period. Clinical information was extracted from the medical records, and is summarized in Table 1.

Morphological review. The materials were fixed in 10% neutral-buffered formalin, embedded in paraffin, cut into 4-μm thick sections, and stained with hematoxylin–eosin (HE) for histologic evaluation. All specimens were reviewed by two pathologists (AMM and YM) to confirm that the morphologic characteristics fulfillled the criteria for FL and DLBCL in the 2001 World Health Organization classification of lymphoid neoplasms.[23] Tumors were judged to be FL grade 1 when neoplastic follicles contained 0–5 centroblasts/10 HPF, and FL grade 2 when they contained 6–15 centroblasts/10 HPF. We diagnosed DLBCL when the tumor cells were spread diffusely without a follicular pattern and large lymphoid cells accounted for more than 30% of the tumor cells. DLBCL was subclassified as the centroblastic, anaplastic, immunoblastic, or T-cell/histiocyte rich variant. The centroblastic variant was subclassified as monomorphous (comprising only large lymphoid cells) or polymorphous (comprising a mixture of large- and medium-sized lymphoid cells).

Immunohistochemistry and *in situ* hybridization. We performed immunohistochemical staining for both FL and DLBCL components on formalin-fixed paraffin-embedded tissues using a panel of monoclonal and polyclonal antibodies. Sections 4-μm thick were cut from each paraffin block, deparaffinized, and incubated at 121°C in pH 6.0 citrate buffer for 10 min for antigen retrieval. Antibodies included those against the following antigens: a pan-B-cell marker, CD20 (L26, ×100; Dako, Glostrup, Denmark); a pan-T-cell marker, CD3 (PS1, ×25; Novocastra, Newcastle-upon-Tyne, UK); FL markers, CD10 (56C6, ×50; Novocastra), Bcl-2 (124, ×100; Dako); and Bcl-6 (poly, ×50; Dako, Kyoto, Japan); postgerminal center B-cell or plasma cell markers, CD30 (Ber-H2, ×100; Dako, Denmark); MUM1 (MUM1p, ×50; Dako, Japan); and CD138 (5F7, ×25; Novocastra); and CD5 (4C7, ×50; Novocastra), employing an autostainer with the standard polymer (Dako autostainer plus: CD3, CD5, CD10, and CD30) or labeled streptavidin-biotin method (Biogenex autostainer: CD20 and Bcl-2), or manually by the standard avidin-biotin complex method (Bcl-6, MUM1, and CD138). Immunoreactivity was judged positive if more than 30% of the tumor cells were stained. All immunohistochemical specimens were judged by AM Maeshima, and Y Matsuno confirmed them.

To classify each case as having either a 'GCB phenotype' or a 'non-GCB phenotype', a panel of three antigens (CD10, Bcl-6, MUM1) was used according to the protocol reported by Hans *et al.*[17] Briefly, cases were assigned to the 'GCB phenotype' if the specimens were positive for CD10. If the specimens were negative for both Bcl-6 and CD10, the corresponding cases were assigned to the 'non-GCB phenotype'. If the specimens were positive for Bcl-6 and negative for CD10, the expression of MUM1 was used to determine the group: if MUM1 was negative, the case was assigned to the 'GCB phenotype', and if positive, to the 'non-GCB phenotype'.

Interphase fluorescence *in situ* hybridization (FISH) analysis. Sections 4-μm thick were cut from each paraffin block and used for FISH analysis. The specimens were treated with a 2× saline sodium citrate buffer (SSC, pH 7.3), digested with 0.005% and 0.3% pepsin/0.01 N HCl for 14 min at 37°C, rinsed in 1× phosphate buffer saline (PBS, pH 7.4) for 5 min, formalin $MgCl_2$/PBS for 10 min, rinsed in 1× PBS for 5 min twice, and dehydrated in an ethanol series. Next, the samples were denatured in 70% formamide/20× SSC for 2 min at 37°C and dehydrated with 70% ethanol for 5 min, followed by 100% ethanol. Denatured probes (10 μL) were dropped onto the slides, covered with a coverslip, and sealed with rubber cement. The slides were then treated using a microwave procedure to intensify the signals. The microwave (MI-77; Azumaya Company, Tokyo, Japan) was set to irradiate the samples for 3-second periods at intervals of 2 s, for a total of 60 min at a frequency of 2.45 GHz and an output power of 250 W with the temperature sensor set to 37°C. After incubation overnight at 37°C, the slides were washed with 50% formamide/2× SSC for 10 min at 45°C, and then washed twice more for 10 min each at room temperature; the slides were then washed with 2× SSC for 10 min. The specimens were rinsed in 4× SSC/0.05% Triton for 5 min, 2× SSC for 5 min at 45°C, and 0.2× SSC at room temperature. The slides were covered with antifade solution and viewed under a BX60 fluorescence microscope (Olympus, Tokyo, Japan) using a 100× oil immersion lens and appropriate filters.

LSI IGH Spectrum Green/LSI BCL2 Spectrum Orange Dual Fusion Translocation Probe (Vysis, Downers Grove, IL, USA) was used to detect t(14;18): IGH/BCL2 fusion. LSI BCL6 Dual Color, Break Apart Rearrangement Probe (Vysis) was used to detect 3q27: BCL6 translocation. Judgment of the fusion gene was performed as described previously.[21] Briefly, a total of 50–200 nuclei per case were scored, and if more than 2% of the tumor cells had two fusion signals in the IGH/BCL2 examination, they were judged positive for fusion. If more than 1.5% of tumor cells had split signals in the BCL6 examination, they were judged positive for translocation.

Statistical analysis. Five-year and 10-year overall survival rates were calculated by the Kaplan–Meier method. Univariate analysis was performed using the log-rank test for clinicopathologic parameters, as shown in Tables 1–3. Clinical information of patients with pre-existing FL and those with coexisting FL was compared by the Fisher's exact test, Mann–Whitney *U*-test or log-rank test in Table 2. Differences were considered significant when *P*-value was less than 0.05.

Results

Patients. Clinical information is summarized in Table 1. The patients comprised 22 men and 21 women, ranging in age from 25 to 80 years with a median age of 53 years. Most of them (34/43) had advanced-stage (III or IV) initially. All of the patients received treatments after initial diagnoses: cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) ± radiation (12 cases), rituximab (R)-CHOP ± radiation (22 cases), or other types of treatment (cyclophosphamide, vincristine, prednisone and procarbazine (C-MOPP), vincristine, cyclophosphamide, prednisone and doxorubicin (VEPA), methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone and bleomycin (MACOP-B), vincristine/vindesine, doxorubicin and prednisone (VCR/VDS + DOX + PSL), R+ radiation and radiation) (8 cases). Overall survival after initial diagnosis was 76.5% at 5 years and 57.3% at 10 years. Overall survival after transformation was 60.0% at 5 years. Clinical information was compared between 19 patients with pre-existing FL and 24 patients with coexisting FL, and is summarized in Table 2.

Morphology. The results of morphological analysis, immunohistochemical staining for each antibody, and FISH analysis are summarized in Table 3 and Table 4. A total of 47 DLBCL specimens from 43 patients were reviewed. The biopsy sites were lymph node (27), tonsil (6), spleen (1), and extranodal sites (13: gingiva 1, stomach 1, small intestine 1, terminal ileum 1, rectum 3, bone marrow 2, skin 3, lung 1). The subtypes of DLBCL in the final specimens were centroblastic monomorphous (30), centroblastic polymorphous (11), and anaplastic (2). Notably, one case (no. 38) finally transformed to classical Hodgkin lymphoma, mixed cellularity, after transformation of FL to DLBCL. Massive necrosis was detected in four cases (9%).

A total of 53 FL specimens from 43 patients were reviewed. The biopsy sites were lymph node (21), tonsil (2), spleen (1), and extranodal sites (29: nasopharynx 1, esophagus 1, stomach 6, duodenum 6, small intestine 2, colon 1, bone marrow 11, skin 1). The grades of FL were grade 1 (20), grade 2 (27), judged as limited to low-grade FL due to the very small amount

Table 1. Patient characteristics

Case	Age/ gender	Stage	IPI	Initial diagnosis (site)	Initial therapy /response	Interval to transformation in months (time of relapse)	Second diagnosis (site)	Subtype of DLBCL	Follow-up months	Outcome from initial diagnosis
1	50/F	2	L	DLBCL + FL, gr.3a + FL, gr.1 (spleen)	Rx3 + CHOPx7/CR	0		anaplastic	40	AWD
2	47/M	2	L	FL, gr.2 (LN)	CHOPx8/CR	91 (2nd)	DLBCL (tonsil)	centroblastic	96	AWD
3	77/M	3	HI	FL, gr.1 (LN)	VCR/VDS, DOX, PSL/unknown	24 (1st)	DLBCL (LN)	centroblastic	31	DOD
4	45/M	3	L	FL, gr.1 (LN)	C-MOPPx11/CR	71 (4th)	DLBCL + FL, gr.3a (LN)	centroblastic	71	AWD
5	44/M	3	L	FL, gr.2 (LN, BM)	R-CHOPx6/PR	69 (1st)	DLBCL + FL, gr.2 (BM)	centroblastic	69	AWD
6	40/F	4	LI	FL, gr.1 (LN)	R-CHOPx6/CR	38 (2nd)	DLBCL + FL, gr.3a (tonsil), DLBCL (rectum), DLBCL (small intestine)	centroblastic	69	AWD
7	66/F	4	LI	FL, gr.1 (LN)	CHOPx3/PR	11 (1st)	DLBCL + FL, gr.3a (LN), DLBCL (skin)	centroblastic	66	AWD
8	80/F	3	HI	DLBCL + FL, gr.3a + FL, gr.2 (LN)	CHOPx5/PR	0		centroblastic	46	DOD
9	51/F	1	L	FL, gr.2 (duodenum)	radiation 40Gy/CR	10 (1st)	DLBCL (LN)	centroblastic	71	AWOD
10	67/M	3	LI	FL, gr.2 (LN)	VEPAx14/CR	93 (1st)	DLBCL (tonsil)	centroblastic	101	DOD
11	60/M	4	LI	DLBCL + FL, gr.2 (small intestine)	CHOPx8 + radiation 39Gy/CR	0		centroblastic	59	AWOD
12	25/F	4	LI	FL, gr.1 (duodenum), DLBCL + FL, gr.3a (LN)	Rx4 + CHOPx8/CR	0		centroblastic	69	AWOD
13	53/M	4	HI	DLBCL + FL, gr.2 (skin)	CHOPx8/PR	0		centroblastic	23	AWD
14	49/M	4	LI	FL, gr.2 (BM)	MACOP–Bx4/PR	49 (2nd)	DLBCL (skin)	centroblastic	55	DOD
15	61/F	3	HI	DLBCL + FL, gr.2 (LN)	Rx4 + CHOPx8/CR	0		centroblastic	44	AWOD
16	46/M	2	L	DLBCL + FL, gr.3a + FL, gr.2 (LN)	R-CHOPx8 + radiation 40Gy/PD	0		centroblastic	19	DOD
17	66/F	3	HI	DLBCL + FL, gr.3a + FL, gr.2 (LN)	CHOPx2 + radiation 40Gy/CR	0		centroblastic	43	AWOD
18	43/M	2	L	DLBCL + FL, gr.1 (LN)	R-CHOPx6 + radiation 40Gy/CR	0		centroblastic	72	AWOD
19	60/F	3	LI	FL, gr.1 (LN, duodenum)	CHOPx8/CR	40 (1st)	DLBCL (tonsil)	centroblastic	80	AWOD
20	41/F	4	LI	FL, gr.2 (tonsil), DLBCL (LN)	R-CHOPx8 + radiation 40Gy/CR	0		centroblastic	38	AWOD
21	72/F	2	LI	FL, gr.2 (LN)	R-CHOPx8 + radiation 40Gy/PR	84 (1st)	DLBCL (LN)	centroblastic	116	AWD
22	46/F	4	H	FL, gr.2 (duodenum, stomach), DLBCL (LN)	R-CHOPx8 + radiation 40Gy/CR	0		centroblastic	32	AWD
23	44/M	3	L	DLBCL + FL, gr.2 (LN)	R-CHOPx8/CR	0		centroblastic	30	AWOD
24	53/F	4	LI	FL, gr.2 (LN)	R + radiation 39Gy/CR	29 (1st)	DLBCL (lung)	centroblastic	29	AWD
25	53/F	4	HI	FL, gr.1 (duodenum, stomach, BM, colon, tonsil), DLBCL + FL, gr.3a (tonsil)	R-CHOPx5/CR	0		centroblastic	33	AWD
26	42/F	4	L	DLBCL + FL, gr.3a (LN), FL, gr.1 (BM)	R-CHOPx6/CR	0		centroblastic	24	AWOD
27	54/M	4	L	FL, gr.2 (LN, BM)	CHOPx8/PR	49 (1st)	DLBCL + FL, gr.3a (BM)	centroblastic	49	AWD

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Table 1. *Continued*

Case	Age/ gender	Stage	IPI	Initial diagnosis (site)	Initial therapy /response	Interval to transformation in months (time of relapse)	Second diagnosis (site)	Subtype of DLBCL	Follow-up months	Outcome from initial diagnosis
28	54/M	4	H	DLBCL + FL, gr.3b + FL, gr.2 (LN)	R-CHOPx8/PR	0		centroblastic	16	AWOD
29	47/M	3	LI	FL, gr.1 (BM)	CHOPx6/CR	46 (2nd)	DLBCL + FL, gr.3b (LN)	centroblastic	47	AWOD
30	59/M	3	L	FL, gr.1 (duodenum), DLBCL + FL, gr.3a (LN)	R-CHOPx6/CR	0		centroblastic	20	AWOD
31	52/M	3	L	DLBCL + FL, gr.3a + FL, gr.2 (LN)	R-CHOPx5/CR	0		centroblastic	18	AWOD
32	57/M	4	L	FL, gr.2 (BM)	R-CHOPx6/CR	26 (1st)	DLBCL + FL, gr.3a (LN)	anaplastic	27	AWD
33	47/F	3	L	DLBCL + FL, gr.1 (LN)	CHOPx6/CR	0		centroblastic	38	AWD
34	47/M	4	LI	FL, low grade (BM), DLBCL (LN)	R-CHOPx8/PR	0		centroblastic	13	AWD
35	47/F	4	L	FL, gr.2 (stomach, BM), DLBCL + FL, gr.3a (LN)	R-CHOPx8/CR	0		centroblastic	7	AWOD
36	59/F	1	L	FL, gr.1 (LN)	CHOPx3 + radiation/CR	24 (1st)	DLBCL (LN)	centroblastic	24	AWD
37	54/M	4	L	FL, low grade (BM), DLBCL + FL, gr.3b (LN)	R-CHOPx6/CR	0		centroblastic	12	AWOD
38	61/F	4	H	FL, low grade (esophagus, stomach), DLBCL + FL, gr.3a (LN), DLBCL (terminal ileum, rectum)	R-CHOPx8/NC	0	HL, MC (LN)	centroblastic	28	DOD
39	68/M	1	L	FL, low grade (BM), DLBCL + FL, gr.3a (LN)	CHOPx3 + radiation/CR	0		centroblastic	8	AWOD
40	79/M	1	L	FL, gr.2 (nasopharynx)	radiation 40Gy/CR	54 (2nd)	DLBCL (stomach)	centroblastic	152	DOD
41	70/M	4	HI	FL, gr.1 (stomach)	Rx4 + CHOPx6/NC	37 (1st)	DLBCL (gingival)	centroblastic	133	DOD
42	45/F	4	L	FL, gr.2 (LN)	C–MOPPx7/CR	21 (1st)	DLBCL (rectum)	centroblastic	37	DOD
43	56/F	4	LI	FL, low grade (stomach), DLBCL (tonsil)	unknown	0		centroblastic	0	unknown

AWD, alive with disease; AWOD, alive without disease; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; C-MOPP, cyclophosphamide, vincristine, prednisone and procarbazine; CR, complete remission; DLBCL, diffuse large B-cell lymphoma; DOD, dead of disease; FL, follicular lymphoma; gr. grade; H, high; HI, high intermediate; HL, MC, Hodgkin lymphoma, mixed cellularity; IPI, international prognostic index; L, low; LI, low intermediate; MACOP-B, methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone and bleomycin; NC, no change; PD, progressive disease; PR, partial remission; R, rituximab; VCR/VDS + DOX + PSL, vincristine/vindesine, doxorubicin and prednisone; VEPA, vincristine, cyclophosphamide, prednisone and doxorubicin.

Table 2. Patients' clinical data at initial diagnosis

	19 cases with pre-existing FL	24 cases with coexisting FL	*P*-value†
Gender (male/female)	11/8	11/13	0.54
Age (median, range)	57 (40–79)	52 (25–80)	0.30
Stage (I, II/III, IV)	5/14	4/20	0.47
LDH (normal/higher than normal)	13/6	14/10	0.54
PS (0/≥1)‡	13/2	13/7	0.24
Extranodal involvement (0/≥1)	13/6	11/13	0.21
B symptom (−/+)‡	14/1	18/2	0.72
Bulky mass (−/+)	18/1	21/3	0.62
IPI (L, LI/HI, H)‡	14/1	14/6	0.10
5-year OS from initial diagnosis	80.2%	65.2%	0.43
5-year OS from transformation	47.7%	65.2%	0.11

†Fisher's exact test, Mann–Whitney *U*-test or log-rank test.
‡data for PS, B symptom, and IPI were not obtained in eight cases.
FL, follicular lymphoma; H, high; HI, high intermediate; IPI, international prognostic index; L, low; LDH, serum lactate dehydrogenase; LI, low intermediate; OS, overall survival; PS, performance status.

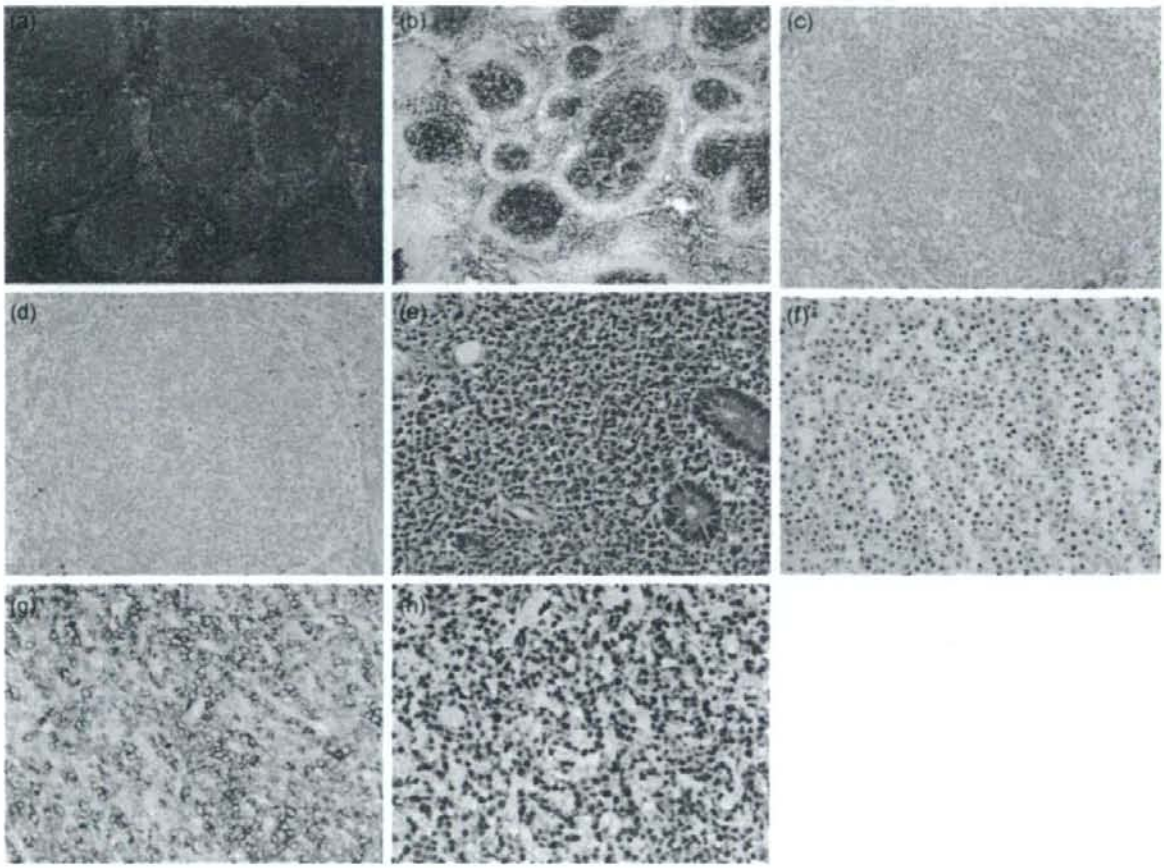

Fig. 1. (a–h) A case of transformation of follicular lymphoma (FL) with a $CD10^+/CD30^-/MUM1^-$ phenotype to diffuse large B-cell lymphoma (DLBCL) with a $CD10^-/CD30^+/MUM1^+$ phenotype. FL, lymph node. (a) Hematoxylin–eosin (HE) (×40), (b) $CD10^+$ (×40), (c) $CD30^-$ (×200), (d) MUM1– (×200). DLBCL, small intestine, (e) HE (×400), (f) $CD10^-$ (×200), (g) $CD30^+$ (×200), (h) $MUM1^+$ (×200).

of material available (6). There were 17 cases with a grade 3a component and three with a grade 3b component.

Immunohistochemistry. The results of immunohistochemistry are summarized in Table 3, Table 4, and Fig. 1. Paraffin sections were available for 41 FL specimens and 43 DLBCL specimens, but in relatively few of the FL and/or DLBCL cases were not available for some of the markers. All tumors were positive for CD20 and negative for CD3. CD10 was positive in 86% (31/36)

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Table 3. Results of immunohistochemistry and FISH analysis

Case no.	Immunophenotype of a low-grade (grade 1 or 2) FL component								FISH[†]		Immunophenotype of a DLBCL component[‡]								FISH		
	CD20	CD10	Bcl-2	Bcl-6	MUM1	CD30	CD5	CD138	IGH/BCL2	BCL6 translocation	CD20	CD10	Bcl-2	Bcl-6	MUM1	CD30	CD5	CD138	GC/non-GC	IGH/BCL2	BCL6 translocation
1	+	+	+	+	−	−	−	−	+		+	+	+	+	−	−	nt	−	GC	+	
2	+	+	+	−	−	nt	−	−	+		+	+	+	+	+	+	+	−	GC	+	
3	+	+	+	+	−	−	nt	−	nt		+	+	+	+	+	−	−	−	GC	+	
4	nt	nt	nt	nt	nt	nt	nt	nt	nt		+	+	+	+	−	−	−	−	GC	+	
5	+	+	+	+	−	−	nt	−	nt		+	nt	+	+	−	−	−	−	GC	nt	
6	+	+	+	+	−	−	−	−	+		+	−	+	−	+	+	−	−	nonGC	+	
7	+	+	+	+	−	−	nt	−	nt		+	−	+	+	+	−	−	−	nonGC	+	
8	+	+	+	+	−	nt	nt	−	+		+	+	+	+	−	−	−	−	GC	+	
9	+	+	+	+	−	−	−	−	+		+	−	nt	nt	nt	nt	nt	nt		+	
10	+	+	+	nt	nt	nt	nt	nt	nt		+	+	−	+	−	−	−	−	GC	+	
11	+	+	+	−	−	−	−	−	nt		+	+	+	−	−	−	−	−	GC	nt	
12	+	+	+	+	+	−	−	−	nt		+	+	+	+	+	−	−	−	GC	−	−
13	+	+	+	+	−	−	nt	−	+		+	+	+	+	−	−	−	−	GC	+	
14	+	nt	+	−	−	−	nt	−	nt		+	+	+	+	−	−	−	−	GC	+	
15	+	+	+	+	−	−	nt	−	+		+	+	+	+	−	nt	−	−	GC	+	
16	+	+	+	+	+	nt	−	nt	+		+	+	+	+	−	nt	−	nt	GC	+	
17	nt	nt	nt	nt	nt	nt	nt	nt	nt		+	+	+	nt	nt	nt	−	nt	GC	nt	
18	+	−	+	+	−	nt	−	nt	nt		+	−	+	+	+	nt	−	nt	nonGC	nt	
19	+	+	+	+	−	−	−	−	nt		+	−	+	nt	nt	nt	nt	nt		nt	
20	+	+	+	+	−	−	nt	−	nt		+	+	+	+	+	−	−	−	GC	+	
21	+	−	−	nt	nt	nt	nt	nt	−	nt	+	−	+	+	−	−	−	−	GC	−	
22	+	+	+	+	−	−	−	−	nt		+	+	+	+	−	−	−	−	GC	+	
23	+	−	+	+	−	−	nt	−	+		+	−	+	nt	nt	nt	−	nt		+	
24	+	+	+	+	−	−	−	−	nt		+	−	+	+	+	+	−	−	nonGC	+	
25	+	+	+	−	+	−	−	−	+		+	+	+	+	+	−	−	−	GC	+	
26	+	+	+	−	−	−	nt	−	+		+	+	+	+	−	nt	nt	nt	GC	+	
27	+	+	+	+	−	nt	nt	nt	+		+	nt	−	+	−	−	−	−	GC	+	
28	+	+	+	nt	nt	nt	nt	nt	+		+	+	+	nt	nt	nt	−	nt	GC	+	
29	+	nt	+	+	+	−	−	−	nt		+	−	+	+	+	+	−	−	nonGC	−	−
30	+	+	+	nt	nt	nt	−	nt	+		+	+	+	+	−	−	−	−	GC	+	
31	+	−	+	nt	nt	nt	nt	nt	nt		+	−	+	nt	−	nt	nt	nt		nt	
32	nt	nt	+	+	+	−	nt	−	nt		+	−	+	nt	+	+	−	nt	nonGC	+	
33	+	+	+	nt	nt	nt	−	nt	nt		+	+	+	nt	nt	−	−	nt	GC	nt	
34	+	+	−,+	+	−	−	−	−	nt		+	+	+	nt	−	nt	−	nt	GC	+	
35	+	+	+	+	−	−	−	−	nt		+	+	+	+	−	−	−	−	GC	+	
36	+	+	+	+	−	−	−	−	nt		+	+	−	+	−	−	−	−	GC	−	−
37	+	nt	+	nt	nt	−	nt	nt	−	nt	+	−	−	nt	nt	nt	−	nt		nt	
38	+	+	+	nt	nt	nt	−	nt	+		+	+	+	−	+	−	−	−	GC	+	
39	+	nt	+	nt	nt	−	nt	nt	nt		+	−	+	+	−	+	−	−	GC	−	+
40	+	+	+	+	−	−	nt	−	nt		+	+	+	−	−	−	−	−	GC	−	−
41	+	+	+	+	−	−	nt	−	nt		+	+	+	+	−	−	−	−	GC	+	
42	+	+	+	+	−	−	−	−	nt		+	−	+	+	−	−	−	nt	GC	nt	
43	+	−	+	nt	nt	nt	−	nt	+		+	+	+	nt	nt	nt	−	nt	GC	+	

[†]judged from the final biopsy specimen, [‡]fluorescence *in situ* hybridization for IGH/BCL2 fusion.
DLBCL, diffuse large B-cell lymphoma; FISH, fluorescence *in situ* hybridization; FL, follicular lymphoma; GC, germinal center B-cell phenotype; nt, not tested.

Table 4. Summary of results of immunohistochemistry and FISH analysis

Antibody	FL component	DLBCL component	Gain/loss/no change
CD20	100% (40/40)	100% (43/43)	
CD10	86% (31/36)*	66% (27/41)†	1/6/29
Bcl-2	96% (42/44)	91% (38/42)	1/4/37
Bcl-6	84% (26/31)	88% (28/32)	4/2/20
MUM1	16% (5/31)	34% (12/35)	7/1/20
CD30	0% (0/28)	20% (6/30)	5/0/16
CD138	0% (0/28)	0% (0/28)	
CD5	0% (0/21)	3% (1/38)	1/0/19
GCB, non-GCB		84% (31/37), 16% (6/37)	
FISH: IGH/BCL2	89% (16/18)	82% (28/34)	

†excluding bone marrow specimens.
DLBCL, diffuse large B-cell lymphoma; FISH, fluorescence *in situ* hybridization; FL, follicular lymphoma; GCB, germinal center B-cell phenotype.

of FLs and 66% (27/41) of DLBCLs, representing a gain in one case, loss in six cases, and no change in 29 cases (including 21 positive cases and eight negative cases). Bone marrow specimens were excluded because only these materials showed extremely low CD10 expression. Among six cases of DLBCL showing loss of CD10, two were diagnosed as DLBCL several times (nos. 6 and 7), and both cases showed loss of CD10 expression between the first and second occasions. Bcl-2 and Bcl-6 were frequently expressed in both FL and DLBCL. Bcl-2 was positive in 96% (42/44) of FLs and 91% (38/42) of DLBCLs, representing a gain in one case, loss in four cases, and no change in 37 cases through transformation. Bcl-6 was positive in 84% (26/31) of FLs and 88% (28/32) of DLBCLs, representing a gain in four cases, loss in two cases, and no change in 20 cases through transformation. Among 32 DLBCL cases for which both Bcl-2 and Bcl-6 immunohistochemistry could be performed, 25 were Bcl-2+/Bcl-6+, four were Bcl-2+/Bcl-6–, 3 were Bcl-2–/Bcl-6+, and no case was Bcl-2–/Bcl-6–.

The postgerminal center B-cell and plasma cell marker MUM 1 was positive in 16% (5/31) of FLs and in 34% (12/35) of DLBCLs, representing a gain in seven cases, loss in one case, and no change in 20 cases (including four positive cases and 16 negative cases). CD30 was negative in all FLs and positive in 20% (6/30) of DLBCLs. Two cases of DLBCL with anaplastic morphology were positive for CD30. CD30 showed scattered expression in marginally and sparsely distributed large lymphoid cells of low-grade FL and FL grade 3, but no case showed positivity in over 30% of the cells. CD5 was negative in all FLs and positive in only one case (no. 2) of DLBCL. This positive case was FL grade 2 in an abdominal lymph node with a $CD10^+$/Bcl-2+/Bcl-6–/$CD5^-$/cyclin D1-immunophenotype and IGH/BCL2 fusion by FISH analysis initially, and showed transformation to centroblastic monomorphous DLBCL in the tonsil, revealing a $CD10^-$/Bcl-2+/Bcl-6+/$CD5^+$/cyclin D1-immunophenotype and IGH/BCL2 fusion by FISH analysis. In the one case of classical Hodgkin lymphoma after transformation from FL via DLBCL (no. 38), FL in the stomach and esophagus and DLBCL in a cervical lymph node had a $CD20^+$/$CD30^-$/$CD10^+$ phenotype and IGH/BCL2 fusion by FISH analysis, but Hodgkin/Reed-Sternberg cells in an inguinal lymph node had a $CD20^-$/$CD30^+$/$CD15^+$/$CD10^-$ phenotype, and were negative for EBER-1 *in situ* hybridization and positive for IGH/BCL2 fusion by FISH (Fig. 2).

Thirty-one (84%) DLBCLs were classified as GCB, and six (16%) DLBCLs were classified as non-GCB. Two cases of DLBCL (nos. 6 and 7) for which several sequential biopsies were taken were judged from the final biopsy specimen: these were non-GCB in the final DLBCL specimens, but had been GCB in the initial specimens.

FISH analysis. Paraffin-embedded sections were available for 18 FL cases and 34 DLBCL cases. IGH/BCL2 fusion was detected in 89% (16/18) of FL cases and in 82% (28/34) of DLBCL cases. In all six DLBCL cases without IGH/BCL2 fusion, BCL6 translocation was detected in one case (17%). Two FL cases without IGH/BCL2 fusion were not available paraffin-embedded sections.

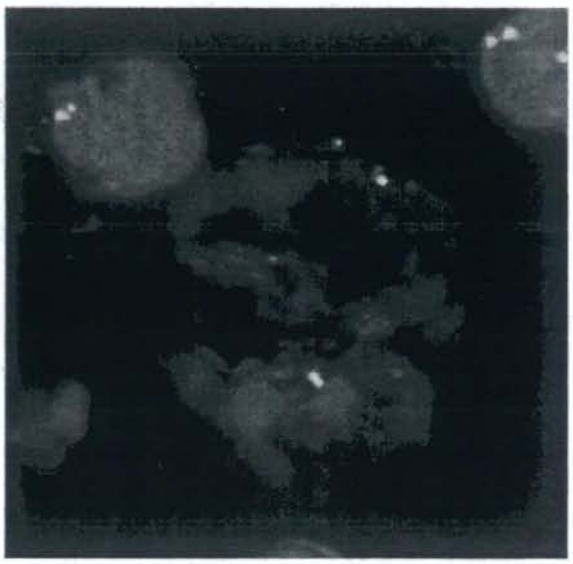

Fig. 2. The result of fluorescence *in situ* hybridization of classical Hodgkin lymphoma transformed from follicular lymphoma (FL). IGH and BCL2 fusion pattern with the LSI IGH Spectrum Green/LSI BCL2 Spectrum Orange Dual Fusion Translocation Probe (Vysis, Downers Grove, IL, USA). Two fusion IGH/BCL2 signals are present.

Statistical analysis. Only the initial treatment regimen was a significant prognostic factor: patients who received CHOP or R-CHOP showed a better outcome than patients who received other treatments ($P < 0.001$). No other significant prognostic factors were detected, including GCB *versus* non-GCB. However, patients with DLBCL showing CD30-positivity (six cases) did not die as a result of disease progression.

Discussion

As transformation of FL to DLBCL is currently the focus of widespread clinical and pathological interests, we studied the

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