thrombus formation occurred, as a CVAS itself is a risk factor for VTE.

However, we did perform DUS at pre-treatment between implantation of the CVAS and induction of bev in a limited number (17) of the patients. The characteristics of these 17 patients showed no differences to those of the other enrolled patients. Of these 17 patients, asymptomatic thrombosis was detected in 5 (29.4%). Of the other 12 patients, 5 showed asymptomatic thrombosis on initial DUS. Treatment with bev was probably associated with thrombus formation in these 5 patients, with incidence lower than that in the total study population (41.7 vs. 53.7%). The characteristics of these 5 patients were also similar to those of the general study population, and their outcomes consisted of a stable thrombus in 3 and asymptomatic progression in 2. The results indicate that a CVAS-associated thrombus prior to induction of bev was not necessarily a significant risk factor for severe thromboembolism.

When comparing the thrombus group with the non-thrombus group, the shorter the time between implantation of CVAS and induction of bev, the greater the risk of thrombus formation, regardless of whether it was symptomatic or asymptomatic. Moreover, a statistically significant difference in thrombus formation was observed between FOLFOX and FOLFIRI (90.9 vs. 9.1%; $P = 0.0047$). However, we do not believe that variation of drugs in FOLFOX versus FOLFIRI was associated with incidence of catheter-related thrombosis, as FOLFOX was used as first-line therapy with implantation of the CVAS, and FOLFIRI as second-line therapy in patients who already had a CVAS. No significant difference in laboratory data was observed between patients receiving FOLFOX and those receiving FOLFIRI; moreover, a shorter time between implantation of the CVAS and induction of bev showed no correlation with poor prognosis of thromboembolism. This point is of particular importance for the physician in treating patients with a bev-based regimen. Therefore, we hypothesized as follows: inhibition of either VEGF or cyclooxygenase (COX)-2-dependent prostacyclin (PGI2) biosynthesis associated with bev may have abolished a tonic protective pathway, thereby increasing the risk of thrombosis. VEGF binds to its major endothelial receptor, kinase insert domain-containing receptor (KDR) or VEGF receptor-2, triggering activation of endothelial nitric oxide synthase (eNOS) and COX-2, enzymes that mediate production of nitric oxide (NO) and PGI2. Bev would interrupt the pathway by which NO and PG12 inhibit platelet aggregation and proliferation of vascular smooth muscle cells, thus increasing risk of thrombosis and arterial wall thickening [29, 30]. Fibroblast growth factor (FGF-2) is quickly released during the wound-healing process, providing an early stimulus for endothelial cell proliferation in the acute phase immediately after injury. FGF-2 appears to be able to up-regulate VEGF production and acts synergistically in stimulating angiogenesis. Platelet-derived growth factor, transforming growth factor-3 and local hypoxia may also regulate VEGF production. Consequently, VEGF increases gradually from the third day after injury onward, providing a sustained stimulus for endothelial cell migration and differentiation into new capillary tubes [31]. Based on these previous reports, we believe that induction of bev in the early phase after implantation of a CVAS may be associated with high risk of thrombus formation due to a low level of VEGF production.

The strength of this study is its prospective assessment of catheter-related thrombus formation using DUS, a highly sensitive and non-invasive strategy. Routine prophylactic anticoagulant treatment at baseline, or if asymptomatic thrombosis was detected, was not permitted; this provided us with the opportunity to evaluate asymptomatic thrombus formation without the influence of prophylactic drugs. The results showed that outcomes in patients with asymptomatic thrombosis mainly depended on changes in thrombus size, as well as decreased vascular flow. In addition, vascular flow appeared to deteriorate with increase in thrombus size.

Our findings indicate that an enlarging thrombus, or large thrombus (>40 mm in diameter), along with decreased venous flow, is a risk factor for symptomatic thromboembolism or PE. Accordingly, we have started to administer prophylactic anticoagulant treatment in such patients at this facility. Further examination of venous flow revealed that thrombi extending into the junction of the SCV, ECV, or SSV strongly affected vascular flow. This finding may furnish an indirect marker of decreased vascular flow.

The American Society of Clinical Oncology provides guidelines on the prevention of recurrent VTE in oncology patients [14]. LMWH is the preferred initial approach for established VTE, and is also preferred in long-term prevention (>6 months). Vitamin K antagonists are an option when LMWH is not available. In Japan, LMWH has not been approved, and unfractionated heparin is used as initial therapy, followed by long-term warfarin therapy with a targeted INR of 2–3.

In conclusion, we propose that routine prophylactic anticoagulant treatment should not be used in patients treated with bev, as bev can increase the risk of bleeding. Therefore, it is important to assess eligibility for bev before treatment and during routine follow-up using available strategies to prevent severe thromboembolism. The results of this study indicate that a period of 1 week or more should be left between introduction of an IP-CVAS to administration of bev to reduce thrombus formation. DUS may offer the optimum strategy for detection of asymptomatic thrombosis in the early cycles of treatment. Moreover,

detection of an enlarging asymptomatic thrombosis developing into the superior vena cava along with decreased vascular flow or extending into the junction of the SCV, ECV, or SSV by DUS may be predictive of subsequent severe symptomatic thromboembolism. Large randomized controlled trials are needed to investigate the mechanism of VTE associated with bev and optimal management of this problem.

Acknowledgments We would like to thank Dr. Madoiwa (Department of Hematology, Jichi Medical University) for excellent pathophysiological advice on the blood coagulation system and Dr. Furuya (Department of Circulatory Organ Internal Medicine, Tokyo Rinkai Hospital) for technical advice on treatment of thromboembolism.

References

1. Kabbinavar FF, et al. Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. J Clin Oncol. 2005;23:3697–705.
2. Kabbinavar F, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol. 2003;21:60–5.
3. Hurwitz H, et al. Bevacizumab plus irinotecan, fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004;350:2335–42.
4. Giantonio BJ, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol. 2007;25:1539–44.
5. Grothey A, et al. Association between exposure to bevacizumab (BV) beyond first progression (BBP) and overall survival (OS) in patients (pts) with metastatic colorectal cancer (mCRC): results from a large observational study (BRiTE). J Clin Oncol. 2007;25(June 20 Supplement):4036. (Abstract).
6. Scappaticci FA, et al. Arterial thromboembolic events in patients with metastatic carcinoma treated with chemotherapy and bevacizumab. J Natl Cancer Inst. 2007;99:1232–9.
7. Nalluri SR, Chu D, Keresztes R, Zhu X, Wu S. Risk of venous thromboembolism with the angiogenesis inhibitor bevacizumab in cancer patients: a meta-analysis. JAMA. 2008;300(19):2277–85.
8. Lee AY, et al. Incidence, risk factors, and outcomes of catheter-related thrombosis in adult patients with cancer. J Clin Oncol. 2006;24:1404–8.
9. Verso M, Agnelli G. Venous thromboembolism associated with long-term use of central venous catheters in cancer patients. J Clin Oncol. 2003;21:3665–75.
10. Montréal M, et al. Pulmonary embolism in patients with upper extremity DVT associated to venous central lines: a prospective study. Thromb Haemost. 1994;72:548–50.
11. Fagnani D, et al. Thrombosis-related complications and mortality in cancer patients with central venous devices: an observational study on the effect of antithrombotic prophylaxis. Ann Oncol. 2007;18:551–5.
12. Couban S, et al. Randomized placebo-controlled study of low-dose warfarin for the prevention of central venous catheter-associated thrombosis in patients with cancer. J Clin Oncol. 2005;23:4063–9.
13. Karthaus M, et al. Dalteparin for prevention of catheter-related complications in cancer patients with central venous catheters: final results of a double-blind, placebo-controlled phase III trial. Ann Oncol. 2006;17:289–96.
14. Khorana AA, Francis CW, Culakova E, Kuderer NM, Lyman GH. American Society of Clinical Oncology guideline: recommendations for venous thromboembolism prophylaxis and treatment in patients with cancer. J Clin Oncol. 2007;25:5490–505.
15. Khorana AA, Francis CW, Culakova E. Thromboembolism is a leading cause of death in cancer patients receiving outpatient chemotherapy. J Thromb Haemost. 2007;5:632–4.
16. Heit JA, et al. Risk factors for deep vein thrombosis and pulmonary embolism: a population-based case-control study. Arch Intern Med. 2000;160:809–15.
17. Miller KD, et al. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. J Clin Oncol. 2005;23:792–9.
18. Johnson DH, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol. 2004;22:2184–91.
19. Vescia S, et al. Management of venous port systems in oncology: a review of current evidence. Ann Oncol. 2008;19:9–15.
20. Newman KA, Reed WP, Schimpff SC, Bustamante CI, Wade JC. Hickman catheters in association with intensive cancer chemotherapy. Support Care Cancer. 1993;1:92–7.
21. Drakos PE, et al. Low molecular weight heparin for Hickman catheter-induced thrombosis in thrombocytopenic patients undergoing bone marrow transplantation. Cancer. 1992;70:1895–8.
22. Lokich JJ, Becker B. Subclavian vein thrombosis in patients treated with infusion chemotherapy for advanced malignancy. Cancer. 1983;52:1586–9.
23. Koksoy C, Kuzu A, Erden I, Akkaya A. The risk factors in central venous catheter-related thrombosis. Aust NZ J Surg. 1995;65:796–8.
24. Cortelezzi A, et al. Incidence of thrombotic complications in patients with haematological malignancies with central venous catheters: a prospective, multicenter study. Br J Haematol. 2005;129:811–7.
25. Verso M, et al. Enoxaparin for the prevention of venous thromboembolism associated with central vein catheter: a double-blind, placebo-controlled, randomized study in cancer patients. J Clin Oncol. 2005;23:4057–62.
26. Berry S et al. Lack of effect of starting bevacizumab shortly after venous access device implantation on wound healing/bleeding complications: preliminary results from first BEAT. ASCO Gastrointestinal Cancer Symposia 2006. San Francisco, CA; January 26–28, 2006. p. 245 (Abstract).
27. Grothey A, et al. Association between exposure to bevacizumab beyond first progression and overall survival in patients with metastatic colorectal cancer: Results from a large observational study (BRiTE). Proc Am Soc Clin Oncol. 2007;172s:4036. (Abstract).
28. Saltz L, et al. Bevacizumab in combination with XELOX or FOLFOX: Updated efficacy results from XELOX-1/N016966, a randomized phase III trial in first line metastatic colorectal cancer. Proc Am Soc Clin Oncol. 2007;170s:4028. (Abstract).
29. Barnard K. Viewpoint: an explanation for the cardiovascular effects of bevacizumab and rofecoxib? Circulation. 2006;114(19):f173–5.
30. Zachary I, Mathur A, Yla-Herttuala S, Martin J. Vascular protection: a novel nonangiogenic cardiovascular role for vascular endothelial growth factor. Arterioscler Thromb Vasc Biol. 2000;20(6):1512–20.
31. Nissen NN, et al. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. Am J Pathol. 1998;152(6):1445–52.

Cancer Science
The official journal of the Japanese Cancer Association
JCA

Phase I study of inotuzumab ozogamicin (CMC-544) in Japanese patients with follicular lymphoma pretreated with rituximab-based therapy

Michinori Ogura,[1,5] Kensei Tobinai,[2] Kiyohiko Hatake,[3] Toshiki Uchida,[1] Masanobu Kasai,[1] Takashi Oyama,[1] Tatsuya Suzuki,[1] Yukio Kobayashi,[2] Takashi Watanabe,[2] Teruhisa Azuma,[2] Masakazu Mori,[2] Yasuhito Terui,[3] Masahiro Yokoyama,[3] Yuko Mishima,[3] Shunji Takahashi,[3] Chiho Ono[4] and Junko Ohata[4]

[1]Department of Hematology and Oncology, Nagoya Daini Red Cross Hospital, Nagoya; [2]Hematology and Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo; [3]Department of Hematology, Cancer Institute Hospital, Tokyo; [4]Medical Research, Wyeth Research, Tokyo, Japan

(Received February 25, 2010/Revised April 12, 2010/Accepted April 18, 2010/Accepted manuscript online April 23, 2010/Article first published online May 19, 2010)

Inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapeutic agent composed of an anti-CD22 antibody conjugated to calicheamicin, a potent cytotoxic antibiotic, specifically targets the CD22 antigen present in >90% of B-lymphoid malignancies, rendering it useful for treating patients with B-cell non-Hodgkin lymphoma (B-NHL). This phase I study evaluated the safety, tolerability, efficacy, and pharmacokinetics of inotuzumab ozogamicin in Japanese patients. Eligible patients had relapsed or refractory CD22-positive B-NHL without major organ dysfunction. Inotuzumab ozogamicin was administered intravenously once every 28 days (dose escalation: 1.3 and 1.8 mg/m^2). All 13 patients had follicular lymphoma, were previously treated with ≥1 rituximab-alone or rituximab-containing chemotherapy, and were enrolled into two dose cohorts (1.3 mg/m^2, three patients; 1.8 mg/m^2, 10 patients). No patient had dose-limiting toxicities, and the maximum tolerated dose, previously determined in non-Japanese patients (1.8 mg/m^2), was confirmed. Drug-related adverse events (AEs) included thrombocytopenia (100%), leukopenia (92%), lymphopenia (85%), neutropenia (85%), elevated AST (85%), anorexia (85%), and nausea (77%). Grade 3/4 drug-related AEs in ≥15% patients were thrombocytopenia (54%), lymphopenia (31%), neutropenia (31%), and leukopenia (15%). The AUC and C_{max} of inotuzumab ozogamicin increased dose-dependently with pharmacokinetic profiles similar to non-Japanese. Seven patients had complete response (CR, 54%) including unconfirmed CR, four patients had partial response (31%), and two patients had stable disease (15%). The overall response rate was 85% (11/13). Inotuzumab ozogamicin was well tolerated at doses up to 1.8 mg/m^2 and showed preliminary evidence of activity in relapsed or refractory follicular lymphoma pretreated with rituximab-containing therapy, warranting further investigations. This trial was registered in ClinicalTrials.gov (NCT00717925). (*Cancer Sci* 2010; 101: 1840–1845)

The successful use of monoclonal antibodies (mAbs) in the treatment of human diseases has been growing steadily in the past decade. Rituximab, a human-mouse chimeric anti-CD20, unconjugated antibody, was approved in 1997 in the USA as the first mAb for antilymphoma therapy. It is now most commonly used in combination with chemotherapy for first and subsequent lines of therapy in B-cell non-Hodgkin lymphoma (B-NHL), such as diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL).[1–6] However, a subgroup of patients does not respond, and early relapses occur in patients with initial response, thus indicating rituximab resistance. This indicates a clear unmet need to explore alternative antibodies non-cross resistant to rituximab as a therapy for B-NHL. One alternative is inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapy agent that specifically targets CD22. Inotuzumab ozogamicin is composed of a recombinant engineered humanized IgG4 anti-CD22 antibody G544 conjugated to calicheamicin, a potent cytotoxic antibiotic derivative.[7]

CD22 is a potential therapeutic target for B-NHL because it is expressed in >90% of B-NHL cells.[8] In addition, CD22 is expressed in mature B cells, but not in their precursor or memory B cells, which may potentially minimize the adverse effect of CD22-targeted treatment on long-term immune function. Moreover, when antibodies bind to the CD22 antigen, the antigen is internalized, that is it is not shed into the extracellular environment.[9]

Both inotuzumab ozogamicin and unconjugated calicheamicin showed potent cytotoxic activity *in vitro* against CD22-positive B cells in preclinical studies.[7] In addition, the unconjugated form of inotuzumab ozogamicin, G544, did not demonstrate any antitumor activity in preclinical studies.[7] Inotuzumab ozogamicin inhibited the growth and the establishment of B-cell lymphomas and induced the regression of large B-cell lymphomas in mouse xenograft models.[7] Furthermore, in preclinical models of disseminated B-NHL in which rituximab was ineffective, treatment with inotuzumab ozogamicin lead to a significant tumor regression and an improvement in survival.[10] This potent cytotoxic activity in preclinical murine models of B-cell lymphomas in which rituximab had failed as a therapeutic agent[11] establishes support for the clinical investigation of inotuzumab ozogamicin for the treatment of CD22-positive B-NHL.

A phase I dose escalation study was previously conducted in the USA and the European Union in patients with relapsed or refractory B-NHL (both FL and DLBCL).[12] In this study, intravenous administration of the drug demonstrated clinical activity in patients with relapsed or refractory B-NHL with clinically manageable thrombocytopenia as the main toxicity. The maximum tolerated dose (MTD) in this non-Japanese patient population was determined to be 1.8 mg/m^2 once every 4 weeks.

The objectives of the present study were to assess the safety, tolerality, efficacy, and pharmacokinetics of inotuzumab ozogamicin in Japanese patients with relapsed or refractory B-NHL who had received prior treatment with rituximab.

[5]To whom correspondence should be addressed.
E-mail: mi-ogura@naa.att.ne.jp
Present affiliations: Teruhisa Azuma, Internal Medicine, Tenri Hospital, Tenri; Junko Ohata, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan.

doi: 10.1111/j.1349-7006.2010.01601.x

Materials and Methods

Study design. The present trial was an open-label multicenter phase I study in which inotuzumab ozogamicin was administered intravenously (IV) as a single agent to patients with CD22-positive B-NHL once every 28 days (±2 days, 1 cycle) for at least four doses provided that the drug was well tolerated with no evidence of progressive disease (PD). The protocol was approved by the Institutional Review Board of each participating institution, and it conformed to the provisions of the Declaration of Helsinki in 1995 (as revised in Tokyo, 2004). All the patients gave written informed consent.

Patients. Patients were eligible for enrollment if they had a diagnosis of CD22-positive B-NHL, according to the World Health Organization (WHO) classification, version 3.[13] Patients were included if they had progressed after at least one prior chemotherapy regimen for indolent B-NHL, or after one or two chemotherapy regiments, which included anthracyline or anthraquinone for aggressive B-NHL. Other inclusion criteria were age ≥20 and <75 years, a performance status of one or better on the Eastern Cooperative Oncology Group Scale, life expectancy ≥12 weeks, an absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L, serum creatinine ≤1.5 × upper limit of normal (ULN), urine protein-to-creatinine ratio of ≤0.2, total bilirubin ≤1.5 × ULN, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 × ULN, and at least one measurable lesion ≥1.5 cm in at least one dimension by computer tomography (CT) at inclusion, in an area of no prior radiation therapy, or clear progression in an area that had been previously irradiated.

Dose escalation and toxicity criteria. Dose escalation decisions were based on the toxicities observed in the first 28 days after the administration of the first dose. Patients (three and 10 patients per cohort) could receive more than the four planned doses of inotuzumab ozogamicin if they experienced at least stable disease and tolerated treatment. The starting dose was 1.3 mg/m^2 administered IV once every 28 days, and dose escalation was performed up to the MTD of 1.8 mg/m^2 administered IV once every 28 days. Both the starting dose and the MTD were based on information from a previous clinical trial.[12] The dose escalation in subsequent cohorts was based on the toxicity assessed in the first 28 days after the first dose. Dose escalation continued until three or more patients in a cohort experienced a dose-limiting toxicity (DLT).

A DLT was defined as any of the following that were at least possibly related to inotuzumab ozogamicin during the first 28 days after the first dose: any grade 3 or 4 (National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTC], version 3.0) nonhematologic toxicity (except grade 3 alopecia, nausea, or vomiting unless the patient was receiving optimal medical therapy); febrile neutropenia (grade 4 ANC ≥3-day duration and temperature ≥38.0°C); grade 4 ANC ≥7-day duration; grade 4 thrombocytopenia ≥3-day duration, or any bleeding episode requiring platelet transfusion; or delayed recovery (to grade 1 or baseline, except alopecia or grade 2 nausea or vomiting unless the patient was receiving optimal medical therapy) from a toxicity related to inotuzumab ozogamicin that delayed the initiation of the next dose by more than 3 weeks. Patients who experienced a DLT had the subsequent doses of inotuzumab ozogamicin reduced by one dose level, the maximum allowed dose reduction per patient. Patients who experienced toxicities other than DLTs could receive additional doses of inotuzumab ozogamicin at the same dose if they met the following criteria: recoveries to ≤grade 1 (nonhematologic), or baseline toxicity except alopecia; ANC $\geq 1.5 \times 10^9$/L; platelet count $\geq 75 \times 10^9$/L; serum creatinine ≤1.5 × ULN, and urine protein-to-creatinine ratio of ≤0.2. The maximum number of doses of inotuzumab ozogamicin was 8 for 1.3 mg/m^2 and 7 for 1.8 mg/m^2.

Pharmacokinetics. Timed blood samples for pharmacokinetic analysis were collected for cycles 1–3 at 0 (pre-dose), 1, 4 (cycles 1 and 3 only), 24, 48, 120, 168, 216, 336, and 504 h relative to the start of infusion for each dosing period and at pre-dose only for cycle 4. If the patient received four doses, then the sample had to be drawn before cycle 5. The serum concentrations of inotuzumab ozogamicin and total calicheamicin were determined using a validated enzyme-linked immunosorbent assay.

The noncompartmental pharmacokinetic parameters of inotuzumab ozogamicin and total calicheamicin were estimated using the WinNonlin (version 4.1) program. The parameters which were determined included the following: end-of-infusion peak concentration (C_{max}), area under the concentration-time curve (AUC), clearance (CL), apparent steady-state volume of distribution (V_{ss}), and the terminal-phase elimination half-life ($t_{1/2}$).

Safety. An AE was considered to be treatment emergent if its onset occurred between the first and the last dose, plus a lag of 28 days provided the following criteria were met: (i) the AE was not present before the start of the first dose and did not occur in the patient as a chronic condition; (ii) the AE was present before the start of the first dose or was part of the patient's medical history, but the severity or frequency increased after the start of the first dose.

Efficacy. Patients were evaluable for efficacy if they received ≥2 doses of inotuzumab ozogamicin, had a baseline tumor CT scan and had undergone at least one tumor assessment for response after baseline assessment. In addition, patients with documented PD prior to receiving two doses of inotuzumab ozogamicin were considered evaluable for efficacy. Tumor response was assessed according to the International Workshop Response Criteria for Non-Hodgkin Lymphoma.[14] The overall response rate (ORR) was defined as the percentage of patients meeting the criteria for complete response (CR), unconfirmed complete response (CRu), or partial response (PR). Stable disease (SD) was measured from the start of the treatment until the criteria for PD were met, taking as the reference the smallest measurements recorded since the initiation of treatment.

Statistical analysis. The sample size for this study was determined by clinical rather than statistical considerations. The probabilities of detecting at least one AE of grade ≥3 with six patients receiving inotuzumab ozogamicin were 0.469, 0.822, and 0.984 when the true rates were 0.10, 0.25, and 0.50, respectively. The probabilities of detecting at least one such event in 10 patients receiving treatment were 0.651, 0.944, and 0.999, respectively.

With cohort sizes of three to six patients, if the true underlying rates of DLT were 0.1, 0.2, 0.3, 0.4, and 0.5, there would be a 0.985, 0.905, 0.754, 0.558, and 0.359 chance, respectively, of escalating to the next full dose. The ORR was estimated using an exact confidence interval (CI) approach.

Results

Patients. From March 2007 to July 2008, a total of 13 patients were enrolled in the study; three patients enrolled in the 1.3 mg/m^2 dose cohort and 10 patients in the 1.8 mg/m^2 dose cohort. The summary of demographic and other baseline characteristics for all patients is presented in Table 1. There were seven males and six females, all with a median age of 49 years (range, 43–72 years). All 13 patients had FL. The median number of prior treatment regimens was 1 (range, 1–13). All 13 patients had previous rituximab treatment (monotherapy or in combination with chemotherapy). Patients were categorized in low (38.5%), intermediate (42%), and high (15%) risk groups according to Follicular Lymphoma International Prognostic Index (FLIPI).[15]

Table 1. Demographic and baseline characteristics, safety population

Characteristics	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
Median age, years (range)	57 (51–66)	48 (43–72)	49 (43–72)
Sex, n (%)			
Female	2 (67)	4 (40)	6 (46)
Male	1 (33)	6 (60)	7 (54)
ECOG performance status, n (%)			
0	3 (100)	10 (100)	13 (100)
Primary diagnosis, n (%)			
Follicular lymphoma	3 (100)	10 (100)	13 (100)
FLIPI risk groups, n (%)			
Low	2 (67)	3 (30)	5 (39)
Intermediate	1 (33)	5 (50)	6 (46)
High	0	2 (20)	2 (15)
Number of prior chemo-/immunotherapy regimens, n (%)			
1	2 (67)	6 (60)	8 (62)
2	0	0	0
3	0	1 (10)	1 (8)
≥4	1 (33)	3 (30)	4 (31)

ECOG, Eastern Cooperative Oncology Group; FLIPI, Follicular Lymphoma International Prognostic Index.

Safety. In dose escalation, no patients had DLTs, and the MTD previously determined in non-Japanese patients (1.8 mg/m^2) was confirmed for Japanese patients in this study. The most common drug-related AEs were thrombocytopenia (100% patients); leukopenia (92%); neutropenia, elevated AST, anorexia, and lymphopenia (85%, each); elevated blood fibrinogen (69%); nausea (77%); elevated ALT, elevated alkaline phosphatase, and decreased hemoglobin (54%, each); malaise, elevated blood bilirubin, and headache (46%, each; Table 2(a)).

A summary of drug-related grade 3 or higher AEs is shown in Table 2(b). At least one drug-related grade ≥3 AEs was reported in nine of the 13 (69%) patients. Drug-related grade ≥3 AEs were thrombocytopenia (7 patients, 54%), lymphopenia and neutropenia (4, 31% each), leukopenia (2, 15%), and elevated blood bilirubin and hypokalemia (1, 8% each). Although neither lymphopenia nor leukopenia was reported for the 1.3 mg/m^2 cohort, the overall incidence of drug-related grade ≥3 AEs was comparable between the two cohorts. There were no patients who died during the study.

A total of four patients experienced dose delays, one (33%) patient in the 1.3 mg/m^2 cohort and three (30%) patients in the 1.8 mg/m^2 cohort (Table 3). Each had one delay. The AEs leading to dose delays were neutropenia (3 patients, 23%) and thrombocytopenia (2, 15%). Two (20%) patients in the 1.8 mg/m^2 cohort had one dose reduction (Table 4). Adverse events (AEs) leading to the dose reduction were thrombocytopenia and pleural effusion (1 patient, 8% each). There were no dose reductions in the 1.3 mg/m^2 cohort.

Seven patients discontinued treatment due to AEs: one patient because of grade 2 rash, one patient because of grade 2 urticaria, and five patients because of AEs that required treatment delays of >3 weeks (two patients with prolonged thrombocytopenia, one patient with prolonged thrombocytopenia and neutropenia, one patient with neutropenia and elevated alkaline phosphatase, and one patient with prolonged neutropenia and elevated total bilirubin).

Pharmacokinetics. Pharmacokinetic data after the first dosing were obtained for all 13 patients. The two patients who received 1.8 mg/m^2 inotuzumab ozogamicin and had a dose reduction after cycle 1 were excluded from pharmacokinetic assessments for cycle 2 and thereafter. The mean ± SD serum concentrations of inotuzumab ozogamicin and total calicheamicin *versus* time

Table 2. Inotuzumab ozogamicin-related adverse events, (a) all grades in ≥4 patients (b) grades ≥3

Adverse event, n (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
(a) all grades in ≥4 patients			
Thrombocytopenia	3 (100)	10 (100)	13 (100)
Leukopenia	3 (100)	9 (90)	12 (92)
Lymphopenia	3 (100)	8 (80)	11 (85)
Neutropenia	3 (100)	8 (80)	11 (85)
Aspartate aminotransferase increased	3 (100)	8 (80)	11 (85)
Anorexia	3 (100)	8 (80)	11 (85)
Nausea	3 (100)	7 (70)	10 (77)
Blood fibrinogen increased	2 (67)	7 (70)	9 (69)
Alanine aminotransferase increased	1 (33)	6 (60)	7 (54)
Blood alkaline phosphatase increased	1 (33)	6 (60)	7 (54)
Hemoglobin decreased	1 (33)	6 (60)	7 (54)
Malaise	3 (100)	3 (30)	6 (46)
Blood bilirubin increased	2 (67)	4 (40)	6 (46)
Headache	2 (67)	4 (40)	6 (46)
Constipation	1 (33)	4 (40)	5 (39)
Influenza	1 (33)	4 (40)	5 (39)
Blood lactate dehydrogenase increased	2 (67)	3 (30)	5 (39)
Fibrin D dimer increased	0	5 (50)	5 (39)
Hyperglycemia	1 (33)	4 (40)	5 (39)
Stomach discomfort	1 (33)	3 (30)	4 (31)
Fatigue	0	4 (40)	4 (31)
Hypercholesterolemia	1 (33)	3 (30)	4 (31)
Hypokalemia	2 (67)	2 (20)	4 (31)
Somnolence	2 (67)	2 (20)	4 (31)
Epistaxis	0	4 (40)	4 (31)
Rash	1 (33)	3 (30)	4 (31)
(b) grades ≥3			
Thrombocytopenia	2 (67)	5 (50)	7 (54)
Lymphopenia	0	4 (40)	4 (31)
Neutropenia	1 (33)	3 (30)	4 (31)
Leukopenia	0	2 (20)	2 (15)
Blood bilirubin increased	1 (33)	0	1 (8)
Hypokalemia	1 (33)	0	1 (8)

Table 3. Number (%) of patients reporting adverse events leading to dose delays, safety population

Parameter, n (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
No. of patients with dose delays			
No dose delays	2 (67)	7 (70)	9 (69)
One or more dose delays	1 (33)	3 (30)	4 (31)
No. of dose delays per patient*			
One	1 (100)	3 (100)	4 (31)
Any adverse event leading to dose delay†	1 (33)	3 (30)	4 (31)
Neutropenia	1 (33)	2 (20)	3 (23)
Thrombocytopenia	1 (33)	1 (10)	2 (15)

*Percentages are based on number of patients with ≥1 inotuzumab ozogamicin dose delay in each treatment group. †Totals at a higher level are not necessarily the sum of those at the lower levels since a patient was able to report two or more different adverse events within the higher level category.

doi: 10.1111/j.1349-7006.2010.01601.x

Table 4. Number (%) of patients reporting adverse events leading to dose reduction, safety population

Parameter, *n* (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (*n* = 3)	1.8 mg/m^2 (*n* = 10)	Total (*n* = 13)
No. of patients with dose reductions			
No dose reductions	3 (100)	8 (80)	11 (85)
One or more dose reductions	0	2 (20)	2 (15)
No. of dose reductions per patient*			
One	0	2 (100)	2 (15)
Any adverse event leading to dose reduction†	0	2 (20)	2 (15)
Thrombocytopenia	0	1 (10)	1 (8)
Pleural effusion	0	1 (10)	1 (8)

*Percentages are based on number of patients with ≥1 dose reduction in each treatment group. †Totals at a higher level are not necessarily the sum of those at the lower levels since a patient was able to report two or more different adverse events within the higher level category.

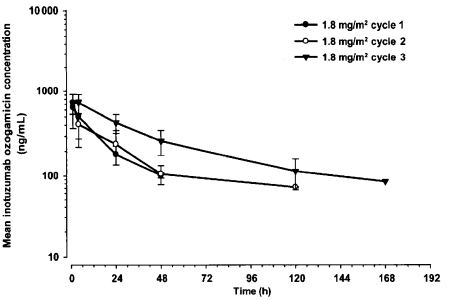

Fig. 1. Mean (SD) serum concentrations of inotuzumab ozogamicin after 1.8 mg/m^2 infusion of inotuzumab ozogamicin once every 4 weeks. Closed circle, cycle 1 (day 1, *n* = 10); open circle, cycle 2 (day 29, *n* = 8); closed triangle, cycle 3 (day 57, *n* = 5).

for patients who received 1.8 mg/m^2 are shown in Figures 1 and 2, respectively. The peak concentration of inotuzumab ozogamicin was generally observed at or shortly after the termination of infusion with moderate intersubject variability. The peak total calicheamicin concentrations were observed typically within 4 h after the start of inotuzumab ozogamicin infusion with small intersubject variability.

The mean pharmacokinetic parameters for inotuzumab ozogamicin and total calicheamicin are shown in Tables 5 and 6, respectively. The AUC of inotuzumab ozogamicin tended to increase with increased dose and period. The $t_{1/2}$ was prolonged with repeated treatment cycles. These were reflected by substantial decreases in clearances.

The mean total calicheamicin C_{max} appeared to increase with dose. The AUC of total calicheamicin increased with increased dose and period. No antibodies to inotuzumab ozogamicin were detectable in patients' serum during the course of the study. The pharmacokinetics data indicate that the disposition of inotuzumab ozogamicin and total calicheamicin following IV treatment was nonlinear with dose or number of doses.

Efficacy. The best tumor response is presented in Table 7. Antitumor activity was observed at both dose levels. In the 1.3 mg/m^2 cohort, two out of three patients had CR, and one patient had CRu for an ORR of 100% (95% CI, 29–100%). In the 1.8 mg/m^2 cohort, one out of 10 patients had CR, three patients had CRu, and four patients had PR for an ORR of 80% (95% CI, 44–98%).

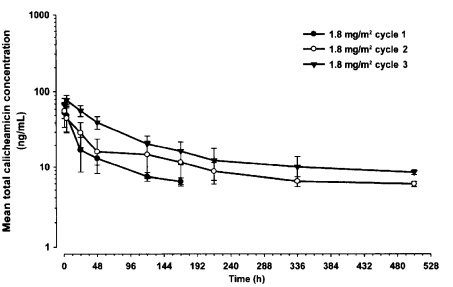

Fig. 2. Mean (SD) serum concentrations of total calicheamicin after 1.8 mg/m^2 infusion of inotuzumab ozogamicin once every 4 weeks. Closed circle, cycle 1 (day 1, *n* = 10); open circle, cycle 2 (day 29, *n* = 8); closed triangle, cycle 3 (day 57, *n* = 5).

Discussion

To improve the clinical outcome of patients with B-NHL who were pretreated with rituximab or rituximab-containing regimens, a number of new agents including antibodies, small mole-

Table 5. Serum pharmacokinetic parameters of inotuzumab ozogamicin

Dose (Once/4 weeks)	Treatment Day (*n*)	Number of cycles	C_{max} (ng/mL) (%)	$t_{1/2}$ (h) (%)	AUC (ng h/mL) (%)	CL (L/h) (%)	V_{ss} (L) (%)
1.3 mg/m^2	1 (3)	1	463 (8)	NC	NC	NC	NC
	29 (3)	2	610 (17)	29.7 (30)	24166 (29)	0.08 (32)	3.27 (11)
	57 (3)	3	524 (18)	43.6 (18)	31642 (21)	0.06 (22)	3.79 (12)
1.8 mg/m^2	1 (10)	1	657 (41)	13.0 (30)	14266 (32)	0.24 (40)	4.06 (21)
	29 (8)	2	727 (27)	35.8 (43)	34518 (46)	0.11 (54)	4.40 (20)
	57 (5)	3	763 (20)	44.0 (32)	39677 (41)	0.09 (56)	4.89 (19)

Data are expressed as mean, and percent coefficient of variance is expressed in parentheses. AUC, total area under the concentration-time curve; CL, clearance; C_{max}, peak concentration; NC, not calculated; $t_{1/2}$, terminal-phase elimination half-life (0.693/λz); V_{ss}, steady-state volume of distribution.

Table 6. Serum pharmacokinetic parameters of total calicheamicin

Dose (Once/4 weeks)	Treatment Day (*n*)	Number of cycles	C_{max} (ng/mL) (%)	$t_{1/2}$ (h) (%)	AUC (ng h/mL) (%)	CL (L/h) (%)	V_{ss} (L) (%)
1.3 mg/m^2	1 (3)	1	44.6 (17)	17.0 (39)	987 (44)	2.35 (58)	49.44 (13)
	29 (3)	2	52.4 (22)	150.6 (45)	5754 (40)	0.38 (48)	62.86 (30)
	57 (3)	3	56.6 (26)	216.3 (55)	8060 (37)	0.27 (43)	60.17 (25)
1.8 mg/m^2	1 (10)	1	59.0 (31)	49.6 (77)	2329 (51)	1.61 (54)	72.3 (28)
	29 (8)	2	59.4 (15)	162.4 (34)	7100 (48)	0.54 (62)	89.18 (41)
	57 (5)	3	78.2 (15)	172.7 (48)	9225 (32)	0.37 (44)	68.37 (26)

Data are expressed as mean, and percent coefficient of variance is expressed in parentheses. AUC, total area under the concentration-time curve; CL, clearance; C_{max}, peak concentration; NC, not calculated; $t_{1/2}$, terminal-phase elimination half-life (0.693/λz); V_{ss}, steady-state volume of distribution.

Table 7. The best tumor response during treatment: number (%) of patients in efficacy population

Best tumor response	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (*n* = 3)	1.8 mg/m^2 (*n* = 10)	Total (*n* = 13)
CR, CRu	3 (100)	4 (40)	7 (54)
PR	0	4 (40)	4 (31)
OR	3 (100)	8 (80)	11 (85)
SD	0	2 (20)	2 (15)

CR, complete response; CRu, unconfirmed complete response; OR, overall response (CR + CRu + PR); PR, partial response; SD, stable disease.

cule, targeted agents, and chemotherapeutic drugs have been developed. However, new treatment modalities with improved toxicity profiles and better responses are needed. Inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapy agent, has demonstrated an acceptable toxicity profile and high activity against relapsed or refractory patients with FL who were pretreated with rituximab or rituximab-containing treatment.

In a recent phase I, multicenter, open-label, dose escalation study of inotuzumab ozogamicin administered IV as a single agent in the USA and the European Union, inotuzumab ozogamicin was found to be reasonably well-tolerated with the MTD of 1.8 mg/m^2 administered every 4 weeks and with the major toxicity of grade 3 or greater thrombocytopenia, which was manageable with careful monitoring and platelet transfusion. Response rates of 69% in patients with FL and 33% in patients with DLBCL in the expanded cohort of this trial were observed.[12]

In the present phase I dose escalation study in Japanese patients with relapsed or refractory FL, who were pretreated with rituximab, the MTD of inotuzumab ozogamicin was determined to be 1.8 mg/m^2 administered once every 28 days, a value that was the same as that observed for non-Japanese patients.

Most common inotuzumab ozogamicin related adverse events were thrombocytopenia, leukopenia, lymphopenia, neutropenia, elevated AST, anorexia, and nausea, a finding that was very similar to the non-Japanese study. Adverse events (AEs) leading to dose delays were neutropenia and thrombocytopenia.

The pharmacokinetic profiles of inotuzumab ozogamicin and total calicheamicin indicated that disposition was non-linear and was associated with increases in drug exposure with increasing dose or number of doses. The pharmacokinetic profiles of inotuzumab ozogamicin and total calicheamicin in Japanese patients were similar to the values for non-Japanese patients. The study population was very limited, thus no definite conclusion can be made for Japanese patients. However, nonlinearities in drug disposition are known for antibodies[16] and had been observed previously for gemtuzumab ozogamicin.[17] Saturable binding with target antigen is thought to influence antibody disposition, potentially leading to nonlinear distribution and elimination.

Potent antitumor activity for inotuzumab ozogamicin was observed at both the 1.3 and 1.8 mg/m^2 dose levels. In the 1.3 mg/m^2 cohort, all three patients had CR or CRu for an ORR of 100%. In the 1.8 mg/m^2 cohort, one out of 10 patients had CR, three patients had CRu, and four patients had PR for an ORR of 80%. Although the number of patients was limited, our preliminary ORR was greater in comparison to other reported antibody-based agents in the treatment of patients with FL and prior exposure to rituximab-containing regimens. For example, in a recent phase I/II study, veltuzumab, a humanized second-generation anti-CD20 monoclonal antibody, was reported to have an ORR of 44%.[18] In another phase I/II, single-agent, dose escalation study, galiximab, an anti-CD80 antibody, demonstrated an ORR of only 11%.[19] Fludarabine phosphate, one of the most effective drugs in the treatment of indolent B-NHL, had an ORR of 65%, when administered as a single agent.[20]

The FLIPI scores in this study were good predictors of favorable outcome. Of the five patients who had low scores (low risk) two demonstrated CR, two had CRu, and one had PR. Of the six patients who had intermediate scores, one had CR, two had CRu, one had PR, and two had SD. The two patients with high FLIPI scores demonstrated only PR.

In conclusion, the results from this phase I study suggest that inotuzumab ozogamicin is safe, well tolerated, and shows promising efficacy in Japanese patients with relapsed or refractory FL pretreated with rituximab-containing therapy. In addition, pharmacokinetics and efficacy in this study are comparable with those in preceding studies in non-Japanese patients. These results therefore warrant further investigation of inotuzumab ozogamicin in relapsed or refractory B-NHL.

Acknowledgments

The authors thank the patients, doctors, nurses and staff members who participated in this multicenter trial for their excellent cooperation. Participating institutions of this phase I study: Nagoya Daini Red Cross Hospital, National Cancer Center Hospital, and Cancer Institute Hospital. Protocol Committee members: Drs Michinori Ogura, Kensei Tobinai, and Kiyohiko Hatake. The authors also thank Joseph Ramcharan, PhD, and Tricia Gooljarsingh, PhD, for writing assistance that was funded by Pfizer, Inc.

Disclosure Statement

This study was funded by Wyeth which was acquired by Pfizer, Inc., in October 2009. Dr. Junko Ohata was an employee of Wyeth K.K. at the time of the study. Dr. Chiho Ono is an employee of Wyeth K.K. No other potential conflict of interest relevant to the article is reported.

doi: 10.1111/j.1349-7006.2010.01601.x

References

1 McLaughlin P, Grillo-López AJ, Link BK *et al.* Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol* 1998; **16**: 2825–33.

2 Czuczman MS, Grillo-López AJ, White CA *et al.* Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J Clin Oncol* 1999; **17**: 268–76.

3 Marcus R, Imrie K, Belch A *et al.* CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. *Blood* 2005; **105**: 1417–23.

4 Hiddemann W, Kneba M, Dreyling M *et al.* Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. *Blood* 2005; **106**: 3725–32.

5 Coiffier B, Lepage E, Briere J *et al.* CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med* 2002; **346**: 235–42.

6 Pfreundschuh M, Trümper L, Osterborg A *et al.* CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. *Lancet Oncol* 2006; **7**: 379–91.

7 DiJoseph JF, Armellino DC, Boghaert ER *et al.* Antibody-targeted chemotherapy with CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. *Blood* 2004; **103**: 1807–14.

8 Leonard JP, Coleman M, Ketas JC *et al.* Epratuzumab, a humanized anti-CD22 antibody, in aggressive non-Hodgkin's lymphoma: phase I/II clinical trial results. *Clin Cancer Res* 2004; **10**: 5327–34.

9 Vaickus L, Ball ED, Foon KA. Immune markers in hematologic malignancies. *Crit Rev Oncol Hematol* 1991; **11**: 267–97.

10 DiJoseph JF, Goad ME, Dougher MM *et al.* Potent and specific antitumor efficacy of CMC-544, a CD22-targeted immunoconjugate of calicheamicin, against systemically disseminated B-cell lymphoma. *Clin Cancer Res* 2004; **10**: 8620–9.

11 DiJoseph JF, Dougher MM, Kalyandrug LB *et al.* Antitumor efficacy of a combination of CMC-544 (inotuzumab ozogamicin), a CD22-targeted cytotoxic immunoconjugate of calicheamicin, and rituximab against non-Hodgkin's B-cell lymphoma. *Clin Cancer Res* 2006; **12**: 242–9.

12 Fayad L, Patel H, Verhoef G *et al.* Clinical activity of the immunoconjugate CMC-544 in B-cell malignancies: preliminary report of the expanded maximum tolerated dose (MTD) cohort of a phase I study. *Blood* 2006; **108**: abs 2711.

13 Harris NL, Jaffe ES, Diebold J *et al.* World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. *J Clin Oncol* 1999; **17**: 3835–49.

14 Cheson BD, Horning SJ, Coiffier B *et al.* Report of an International Workshop to Standardize Response Criteria for Non-Hodgkin's Lymphomas. *J Clin Oncol* 1999; **17**: 1244–53.

15 Solal-Céligny P, Roy P, Colombat P *et al.* Follicular lymphoma international prognostic index. *Blood* 2004; **104**: 1258–65.

16 Lobo ED, Hansen RJ, Balthasar JP. Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci* 2004; **93**: 2645–68.

17 Dowell JA, Korth-Bradley J, Liu H, King S, Berger MS. Pharmacokinetics of gemtuzumab ozogamicin, an antibody-targeted chemotherapy agent for the treatment of patients with acute myeloid leukemia in first relapse. *J Clin Pharmacol* 2001; **41**: 1206–14.

18 Morschhauser F, Leonard JP, Fayad L *et al.* Humanized anti-CD20 antibody, veltuzumab, in refractory/recurrent non-Hodgkin's lymphoma: phase I/II results. *J Clin Oncol* 2009; **27**: 3346–53.

19 Czuczman MS, Thall A, Witzig TE *et al.* Phase I/II study of galiximab, an anti-CD80 antibody, for relapsed or refractory follicular lymphoma. *J Clin Oncol* 2005; **23**: 4390–8.

20 Tobinai K, Watanabe T, Ogura M *et al.* Phase II study of oral fludarabine phosphate in relapsed indolent B-cell non-Hodgkin's lymphoma. *J Clin Oncol* 2006; **24**: 174–80.

blood

2010 116: 5631-5637
Prepublished online Sep 9, 2010;
doi:10.1182/blood-2010-06-290650

Lymphomatoid gastropathy: a distinct clinicopathologic entity of self-limited pseudomalignant NK-cell proliferation

Kengo Takeuchi, Masahiro Yokoyama, Shin Ishizawa, Yasuhito Terui, Kimie Nomura, Kousuke Marutsuka, Maki Nunomura, Noriyasu Fukushima, Takahiro Yagyuu, Hirokazu Nakamine, Futoshi Akiyama, Kazuei Hoshi, Kosei Matsue, Kiyohiko Hatake and Kazuo Oshimi

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

Lymphomatoid gastropathy: a distinct clinicopathologic entity of self-limited pseudomalignant NK-cell proliferation

Kengo Takeuchi,[1,2] Masahiro Yokoyama,[3] Shin Ishizawa,[4] Yasuhito Terui,[3] Kimie Nomura,[2] Kousuke Marutsuka,[5] Maki Nunomura,[6] Noriyasu Fukushima,[7] Takahiro Yagyuu,[8] Hirokazu Nakamine,[9] Futoshi Akiyama,[2] Kazuei Hoshi,[10] Kosei Matsue,[11] Kiyohiko Hatake,[3] and Kazuo Oshimi[12]

[1]Pathology Project for Molecular Targets and [2]Division of Pathology, The Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan; [3]Division of Hematology, The Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, Japan; [4]Department of Pathology, Faculty of Medicine, University of Toyama, Toyama, Japan; [5]Pathology Division, Miyazaki Medical College Hospital, University of Miyazaki, Miyazaki, Japan; [6]Department of Pathology, Tachikawa Sougo Hospital, Tokyo, Japan; [7]Department of Internal Medicine, Faculty of Medicine, Saga University, Saga, Japan; [8]Department of Diagnostic Pathology, School of Medicine, Nara Medical University, Nara, Japan; [9]Department of Immunopathology, Kansai University of Health Sciences, Nara, Japan; [10]Department of Pathology, Kameda General Hospital, Chiba, Japan; [11]Division of Hematology/Oncology, Department of Medicine, Kameda General Hospital, Chiba, Japan; and [12]Eisai Research Institute of Boston, Andover, MA

Diagnostic errors in distinguishing between malignant and reactive processes can cause serious clinical consequences. We report 10 cases of unrecognized self-limited natural killer–cell proliferation in the stomach, designated as lymphomatoid gastropathy (LyGa). This study included 5 men and 5 women (age, 46-75 years) without any gastric symptoms. Gastroscopy showed elevated lesion(s) (diameter, ~ 1 cm). Histologically, medium-sized to large atypical cells diffusely infiltrated the lamina propria and, occasionally, the glandular epithelium. The cells were CD2$^{+/-}$, sCD3^{-}, cCD3^{+}, CD4^{-}, CD5^{-}, CD7^{+}, CD8^{-}, CD16^{-}, CD20^{-}, CD45^{+}, CD56^{+}, CD117^{-}, CD158a^{-}, CD161^{-}, T cell–restricted intracellular antigen-1^{+}, granzyme B^{+}, perforin^{+}, Epstein-Barr early RNA^{-}, T-cell receptor $\alpha\beta^{-}$, and T-cell receptor $\gamma\delta^{-}$. Analysis of the 16 specimens biopsied from 10 patients led to a diagnosis of lymphoma or suspected lymphoma in 11 specimens, gastritis for 1 specimen, adenocarcinoma for 1 specimen, and LyGa or suspected LyGa for 3 specimens. Most lesions underwent self-regression. Three cases relapsed, but none of the patients died. According to conventional histopathologic criteria, LyGa is probably diagnosed as lymphoma, especially as extranodal natural killer/T-cell lymphoma, nasal type. However, LyGa is recognized as a pseudomalignant process because of its clinical characteristics. The concept of LyGa should be well recognized. (*Blood.* 2010;116(25):5631-5637)

Introduction

The World Health Organization classification of tumors of hematopoietic and lymphoid tissues lists > 60 types of lymphomas.[1] Several reactive or borderline lesions related to these overt lymphomas are well known. Some benign lymphoproliferative disorders, including infectious mononucleosis, drug-induced lymphadenitis especially related to anticonvulsants, and histiocytic/subacute necrotizing lymphadenitis (Kikuchi-Fujimoto disease),[2,3] are occasionally misdiagnosed as malignancy because these lesions histopathologically mimic lymphoma.[4] They are basically self-limited and require no cytoreductive therapies. Lymphomatoid papulosis, lymphomatoid granulomatosis, and methotrexate-associated lymphoproliferative disorder[5] are listed as borderline lesions with uncertain malignant potential according to the World Health Organization. These disorders may also be diagnosed as overt lymphoma. Moreover, even if they are properly diagnosed, selection of a treatment strategy is then a matter of discussion because some of these cases undergo spontaneous regression. Therefore, conservative therapies are primarily favored in such cases, and these lesions should be treated as lymphoma only if they are clinically malignant. In any case, at the time these lesions are evaluated with biopsy specimens, the possibility of being benign should be well considered, and overtreatment must be carefully avoided.

Here, we report 10 cases of a pseudomalignant disorder caused by an unrecognized atypical natural killer (NK)–cell proliferation in the stomach; we have designated this disorder as lymphomatoid gastropathy (LyGa). According to conventional histopathologic criteria, such lesions are diagnosed as lymphoma, especially as extranodal NK/T-cell lymphoma, nasal type. However, considering its clinical characteristics, LyGa is recognized as a pseudomalignant process because it spontaneously regresses without any treatment.

Methods

Patients

During the 11-year period between 1998 and 2009, there were 10 cases of CD56-positive atypical lymphoid cell proliferation in the stomach (patients 1-3 presented at the Cancer Institute and patients 4-10 were referred to K.T. for consultation). The clinical records and pathology materials of the cases were reviewed.

Submitted June 15, 2010; accepted August 31, 2010. Prepublished online as *Blood* First Edition paper, September 9, 2010; DOI 10.1182/blood-2010-06-290650.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

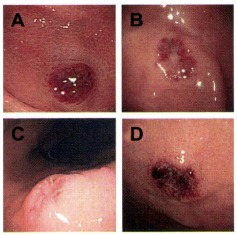

Figure 1. Gross appearance of LyGa. Cases 3 (A), 3 (B), 4 (C), and 10 (D) are shown.

Immunophenotyping and Epstein-Barr virus detection

Immunohistochemical examination was performed with Autostainer (Dako); dextran-polymer method (EnVision+; Dako); and antibodies against CD2, CD3, CD4, CD5, CD7, CD8, CD20, CD30, CD45, CD56, CD68 (KP1 or PGM1), T cell–restricted intracellular antigen-1 (TIA1), granzyme B, anaplastic lymphoma kinase, myeloperoxidase, Ki67, and T-cell receptor βF1 (TCRβF1). For flow cytometry, the following antibodies were used: CD2, CD3, CD7, CD56, TCRαβ, TCRγδ, TCRVa24, CD158a, and CD161. The presence of Epstein-Barr virus (EBV) was assessed by in situ hybridization for Epstein-Barr early RNA (EBER).

Polymerase chain reaction analysis for TCRγ gene rearrangement

DNA was extracted from the paraffin sections with the use of Recover All Total Nucleic Acid Isolation according to the manufacturer's instructions (Ambion). A seminested protocol involving 2 rounds of polymerase chain reaction (PCR) was used for the amplification of the rearranged TCRγ gene with the use of the primers TVγ, 5′-AGGGTTGTGTTGGAATCAGG-3′; TJγ-out, 5′-CGTCGACAACAAGTGTTGTTCCAC-3′; and TJγ-in, 5′-GGATCCACTGCCAAAGAGTTTCTT-3′. The 5′ end of TJγ-I was labeled by cyanine 5 for fragment analysis. In all the experiments, monoclonal (Jurkat cells) and polyclonal (placental tissue from a healthy person) controls were run in parallel with the samples. The PCR products were analyzed with CEQ8000 (Beckman Coulter Inc). DNA from each sample was amplified ≥ 6 times.

Results

Clinical history

Of the 10 patients in this study, 5 were men and 5 were women. The age of these patients ranged from 46 to 75 years Three patients had a history of gastric cancer, of whom 1 had previously undergone endoscopic mucosal resection 2 times (case 1) and the other 2 had previously undergone partial gastrectomy (cases 3 and 8). At the time of the study, 3 patients had diabetes mellitus (cases 1, 2, and 9) and 4 had hypertension (cases 2, 7, 9, and 10). Blood cell counts and chemistry, including lactic dehydrogenase levels, were within the normal limits in all patients. There were no gastric symptoms at the time of gastroscopy. The 3 patients with history of gastric cancer underwent gastroscopy during a follow-up study for gastric cancer, and the procedure was performed on the other patients as a secondary checkup because gastric x-ray screening for cancer in these patients showed the presence of abnormal shadows. Gastroscopy showed ulcerative or elevated lesion(s) ~ 1 cm in diameter in the stomach (Figure 1A-D). The pathologists of the institutions where the biopsies of the patients with LyGa were first performed diagnosed the patients' conditions as lymphoma or suspected lymphoma (cases 1, 2, 5-8, and 10), gastritis with histiocytic infiltration (case 3), and poorly differentiated adenocarcinoma (case 4). In case 3, the specimen was biopsied again 11 months later, and the patient's condition was then diagnosed as NK/T-cell lymphoma. Cases 5 and 9 were suspected of having lymphoma, and the pathologist consulted with one of the authors (K.T.), leading to the diagnosis of LyGa. In case 5, another biopsy was performed 3 weeks after the first biopsy for flow cytometry.

An extensive workup, including ultrasonography (cases 1-4 and 9), computed tomographic (cases 1-4 and 6-9), and 2-[fluorine-18]fluoro-2-deoxy-D-glucose positron emission tomographic scans (cases 2, 4, 6, and 8); colonoscopy (cases 2, 4-6, and 9); and bone marrow biopsy (cases 1-4 and 7-9), was performed. The results showed no evidence of lymphoma in sites other than the stomach. Multiple serologic studies for celiac disease showed no evidence of high titers of anti–gliadin immunoglobulin A and immunoglobulin G antibodies in cases 2 and 4. Gastroscopy and biopsy were performed 1-4 months after the biopsies, which showed no evidence of lymphoma (cases 1, 2, 5, 7 and 10). Cases 4 and 6 underwent partial gastrectomy 1 month after the initial biopsy diagnosis, resulting in no evidence of carcinoma or lymphoma. All the patients were carefully watched and followed up without chemotherapy. Except in the case of patients 3, 8, and 9, none of the other patients had any recurrences. In case 3, the patient developed 3 lesions; on follow-up examination 11 months later, the lesions had regressed, and a new lesion was detected. The new lesion also regressed in 1 month from the second biopsy. In case 8, the patient developed another lesion 7 months after self-regression of the first lesion; this new lesion also regressed in 3 months without any treatment. In case 9, the first lesion could not be detected 4 months from the first biopsy; however, 2 new lesions were detected. After another 4 months, these 2 lesions could also not be detected, and 2 new lesions were identified. The consequence of the 2 lesions last detected is unknown because the patient refused further gastroscopic examination.

Morphology

Grossly, the lesions were flat elevations with or without a shallow depression and were approximately 1 cm in diameter (Figure 1A-D). The atypical cells diffusely infiltrated the lamina propria and occasionally into the glandular epithelium (Figure 2A), simulating the lymphoepithelial lesion seen in extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue lymphoma, which was designated as lymphoepithelial-like lesion by NK cells (Figure 2B). In some cases, necrosis was present, but there were no angiocentric or angiodestructive growth patterns or apoptotic bodies. Mitotic figures were occasionally present. The atypical cells were medium to large with moderate to abundant clear or slightly eosinophilic cytoplasm. The nuclei were generally round to oval, but some were irregular and indented, with fine chromatin and a few inconspicuous nucleoli. These cytomorphologic features somewhat give a histiocyte-like impression. Interestingly, specimens for all the patients contained a variable proportion of cells (20%-90%) with eosinophilic granules in the cytoplasm (Figure 2C-D). In some cases, atypical cells with a prominent nucleolus were observed (Figure 2D). Small reactive lymphocyte aggregates and neutrophils may be occasionally found. Nine of the patients had *Helicobacter pylori* infection.

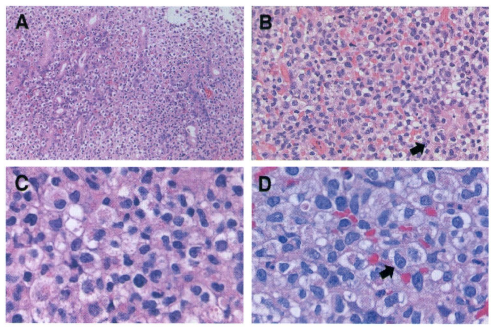

Figure 2. Histopathology of LyGa. The pattern of infiltration is diffuse (A; case 1; 20× objective). Atypical NK cells occasionally infiltrate the glandular epithelium (arrow), showing lymphoepithelial-like lesions by NK cells (B; case 10; 40× objective). Some atypical cells harbor large eosinophilic granules in the cytoplasm (C; case 3; 100× objective). In some cases, the nucleoli are prominent (arrow; D; case 5; 100× objective). Figures were taken with a microscope (BX51; Olympus) and a digital camera (KY-F75; Victor). Microsoft PowerPoint 2007 was used for image processing. Numeric apertures: 20×/0.40 (A), 40×/0.75 (B), 40×/0.95 (C), 60×/0.90 (D).

Immunophenotype and EBER in situ hybridization

The atypical cells were strongly positive for CD7, CD56, and cytotoxic molecule-associated proteins (TIA1, granzyme B, and perforin; Figure 3A-C). CD2 and CD45 were variably positive. CD3ε was positive in the cytoplasm, but a membrane-staining pattern was not observed (Figure 3D). Anaplastic large cell lymphoma-associated markers (CD30 and anaplastic lymphoma kinase) were negative. Other common lineage markers, including B-cell (CD20), T-cell (CD4, CD5, and CD8), and myelomonocytic (CD68 and myeloperoxidase) markers, were all negative. EBER in situ hybridization was negative. The results of immunohistochemistry for individual cases are listed in Table 1. For case 5, flow cytometric analysis was performed with the second specimen, which was obtained from a biopsy performed 3 weeks after the first biopsy (Figure 4). Grossly, although the lesion was regressing, it remained present. The atypical cells of this case expressed CD7 and CD56 (both aberrantly bright) and CD2 (negative or dim). Other T or NK cell–related markers were negative (CD3, CD16, TCRαβ, TCRγδ, TCRVa24, CD158a, and CD161).

PCR analysis for TCRγ gene rearrangement

PCR analysis for TCRγ gene rearrangement was performed 6 times per case for cases 1-4 and 8. No reproducible rearranged bands were observed (data not shown).

Discussion

Here, we report 10 cases of self-limited lymphoma-like lesions in the stomach, which we designated as LyGa. These cases were almost identical to each other in morphology and immunophenotype of atypical cells. Gross examination showed that the lesions were ulcers or flat elevations with a shallow depression, measuring approximately 1 cm in diameter. Microscopic observation showed that they were composed of sheets of large peculiar cells that showed indented nuclei and clear cytoplasm with eosinophilic granules. Immunohistochemical analysis of the atypical cells of LyGa showed that they were $CD2^-$ or variably $CD2^+$, $CD3^+$ (cytoplasmic), $CD4^-$, $CD5^-$, $CD7^+$, $CD8^-$, $CD16^-$, $CD20^-$,

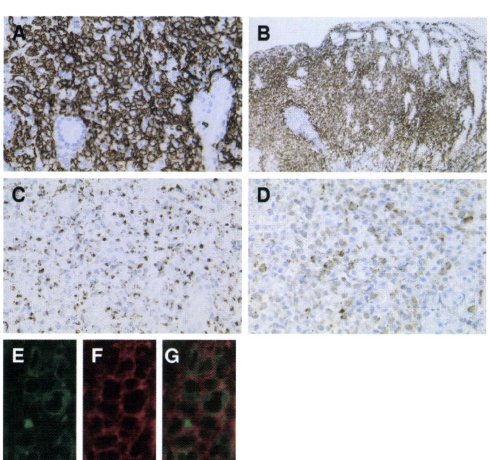

Figure 3. Immunophenotype of LyGa by immunohistochemistry. The atypical cells are positive for CD7 (A; case 5), CD56 (B; case 3), granzyme B (C; case 4), and cytoplasmic CD3ε (D; case 2). To confirm the cytoplasmic localization of CD3ε, fluorescein double immunohistochemistry for CD3ε (E) and CD56 (F) was performed (case 10). In the merged figure (G), the cytoplasmic localization of CD3ε is clearly shown, indicating that the atypical cells are of NK lineage. Figures were taken with a microscope (BX51; Olympus) and a digital camera (KY-F75; Victor). Microsoft PowerPoint 2007 was used for image processing. Numeric apertures: 40× (A,C,D), 10× (B), 60× (E-G).

Table 1. Patient characteristics and immunologic markers

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Age, y	52	58	51	50	55	46	65	56	59	75
Sex	Male	Male	Male	Female	Male	Male	Female	Female	Female	Female
Past history	Two individual early gastric cancers at the ages of 48 and 51 y	NP	Advanced gastric cancer at the age of 47 y	NP	NP	NP	NP	Advanced gastric cancer at the age of 52	NP	NP
H pylori	–	+	+	+	+	+	+	+	+	+
Original pathologic diagnosis	NK/T-cell lymphoma	NK/T-cell lymphoma	Gastritis with histiocytosis. NK/T-cell lymphoma*	Adenocarcinoma	Lymphoma, s/o LyGa*	NK/T-cell lymphoma; NK/T-cell lymphoma*	T-cell lymphoma	T-cell lymphoma; NK/T-cell lymphoma*	Lymphoma, s/o LyGa, s/o LyGa*	T-cell lymphoma
Follow-up examinations, days from the initial biopsy	45, 73, 276, 577, 1165	55, 239, 442, 675, 896, 1121, 1497	336,† 365, 484, 701, 1065, 1429, 1793	41,‡ 167, 1360	13,† 132	50,† 56‡	38, 81, 137, 207, 361, 515, 742, 1029, 1281, 1515	98, 154, 236,† 256, 333, 452, 585, 790	113,† 232†	30, 59, 143, 232, 354
Patient status	Well at 145 mo	Well at 50 mo	Well at 60 mo	Well at 46 mo	Well at 33 mo	Well at 60 mo	Well at 56 mo	Well at 29 mo	Well at 18 mo	Well at 12 mo
Treatment	Observation	Observation	Observation	Subtotal gastrectomy	Observation	Total gastrectomy	Observation	Observation	Observation	Observation
CD2	–	+w	+w	+w	–	+	+w	–	+	+
CD3	+	+	+	+	+	+	+	+	+	+
CD4	ND	–	–	–	–	–	–	–	–	–
CD5	–	–	–	–	–	–	–	–	–	–
CD7	+	+	+	+	+	+	+	+	+	+
CD8	ND	–	–	ND	–	–	–	–	–	–
CD20	–	–	–	–	–	–	–	–	–	–
CD56	+	+	+	+	+	+	+	+	+	+
Cytotoxic molecules	TIA1$^+$	TIA1$^+$, granzyme B$^+$, perforin$^+$	TIA1$^+$, granzyme B$^+$, perforin$^+$	TIA1$^+$, granzyme B$^+$, perforin$^+$	Granzyme B$^+$	TIA1$^+$, Perforin$^+$	Perforin$^+$	TIA1$^+$	Granzyme B$^+$	Granzyme B$^+$
EBER	–	–	–	–	–	–	–	–	–	–
Other markers	CD16$^-$, betaF1$^-$	CD16$^-$, CD30$^-$, CD45$^+$w, CD57$^-$, CD68$^-$, CD123$^-$, betaF1$^-$, ALK$^-$, MPO$^-$, MIB1 index 10%	CD16$^-$, CD30$^-$, CD45$^+$w, CD57$^-$, CD68$^-$, CD123$^-$, betaF1$^-$, ALK$^-$, TdT$^-$, MIB1 index 30%	CD16$^-$, CD45$^+$, CD68$^-$, CD123$^-$, betaF1$^-$, CD43$^+$	TCR$\alpha\beta^-$, TCR$\gamma\delta^-$, CD161$^-$, TCRVα24$^-$, CD16$^-$, CD158a$^-$				CD10$^-$, CD21$^-$, BCL2$^+$, CD45RO$^+$, CD68$^-$, MIB1 index 20%	MIB1 index 20%, CD21$^-$

NP indicates nothing in particular; s/o, suspected of; ND, not done; and +w, weakly positive.

*In case 3, 5, 6, 8 and 9, multiple biopsies showed the presence of LyGa.

†LyGa is present on follow-up examination.

‡In follow-up examinations, days of gastrectomy or gastroscopy with or without biopsy from the initial biopsy are described.

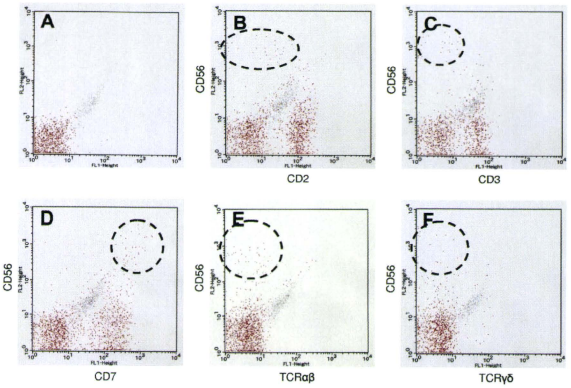

Figure 4. Immunophenotype of LyGa by flow cytometry. Flow cytometry was performed for case 5. The atypical cells were $CD56^{bright}$, $CD2^{dim}$ (B), $CD3^-$ (C), $CD7^{bright}$ (D), $TCR\alpha\beta^-$ (E), and $TCR\gamma\delta^-$ (F). (A) Negative control.

$CD45^+$, $CD56^+$, $CD117^-$ and positive for cytotoxic molecule-related proteins ($TIA1^+$, granzyme B^+, and $perforin^+$). This immunophenotype is highly suggestive of extranodal NK/T-cell lymphoma of the nasal type, which usually arises in extranodal sites, especially in the nasal cavity.[1,6,7]

Extranodal NK/T-cell lymphoma of the nasal type is rarely seen in Western countries and is more common in Asia and in Central and South American countries.[1,6,7] It accounts for ~ 2%,[8] 6%,[9] 8%,[10] and 5%[11] of all newly diagnosed lymphoma cases in Japan, Hong Kong, Korea, and Taiwan, respectively. Histologically, the lymphoma often has an angiocentric and angiodestructive infiltrate of atypical lymphocytes of various sizes leading to extensive necrosis.[1] The immunophenotype of neoplastic cells usually indicates that they are of NK-cell lineage (surface $CD3^-$, cytoplasmic $CD3^+$, $CD5^-$, and $CD56^+$) but are occasionally of T-cell lineage by definition.[1] In previous studies, neoplastic cells in almost all the cases were found to be infected by EBV.[12,13] In localized diseases, the survival rate has recently improved with a combination of upfront radiotherapy and chemotherapy, whereas almost all patients with extensive disease die within a year after diagnosis.[14-16]

Of the 16 biopsied specimens in this study, 11 were diagnosed with lymphoma or suspected lymphoma. Fortunately, however, LyGa has several characteristic features that are not consistent with extranodal NK/T-cell lymphoma. First, the stomach is not a common site of origin in the case of NK/T-cell lymphoma. To the best of our knowledge, there are 10 reported cases of extranodal NK/T-cell lymphoma involving the stomach, and the lesions were not limited to the stomach in any of these cases.[17-21] Second, although some of the cases of LyGa showed necrosis, but angiocentric or angiodestructive growth patterns, and prominent apoptotic bodies, which are common features of extranodal NK/T-cell lymphoma,[1] were not observed. Third, LyGa may show epithelial invasion, that is, lymphoepithelial-like lesion by NK cells. Fourth, the cytomorphology of LyGa is atypical for extranodal NK/T-cell lymphoma. Although the cytologic spectrum of extranodal NK/T-cell lymphoma is broad,[1] to the best of our knowledge, large eosinophilic cytoplasmic granules seen in the atypical cells of LyGa have never been observed in the histopathology section of extranodal NK/T-cell lymphoma although finer granules can often be seen in Giemsa-stained cytologic preparations. Finally, EBER in situ hybridization, which is almost always positive in NK/T-cell lymphoma of the nasal type,[1,12,13] is consistently negative in LyGa. In addition, a differential diagnosis of $CD56^+$ T-cell neoplasm with extensive loss of T-cell markers may be considered. In particular, the immunophenotype of LyGa overlaps the immunophenotype observed in some cases of enteropathy-associated T-cell lymphoma (type II).[22] However, the negative PCR results for the TCR-γ gene rearrangement (performed in cases 1-4 and 8; data not shown) were inconsistent with results obtained for T-cell lymphomas.

Vega et al[23] reported a similar case of atypical NK-cell proliferation probably related to gluten sensitivity mimicking NK-cell lymphoma. In that study, the 32-year-old male patient was positive for anti–gliadin antibody and had persistent multiple lesions in the stomach, small bowel, and large bowel for 3 years.[23] Two of our 10 patients were tested and found to be negative for anti–gliadin antibodies. Actually, gluten intolerance and celiac disease are extremely rare in Japan. However, the immunophenotype and morphology of the atypical cells of our patients were similar to those observed in the case of the 32-year-old man reported by Vega et al.[23] In addition, our cases shared a significant clinical feature with the case reported by Vega et al,[23] that is, "self regression." The lesions of the 32-year-old man persisted for 3 years until he was placed on a gluten- and lactose-free diet, whereas the lesions of our patients did not seem to persist for such an extended period of time. Furthermore, none of our patients were found to have intestinal lesions. These differences might be due to the different stimulants, if any, although we were unable to identify any stimulant(s) in our cases.

Two types of gastric malignant neoplasms, namely, adenocarcinoma and mucosa-associated lymphoid tissue lymphoma, are related to *H pylori* infection. Nine of the 10 cases were positive for *H pylori* infection, and 3 of the patients had a history of gastric adenocarcinoma. Normal NK cells were present in both *H pylori*–infected and uninfected gastric mucosa at approximately 6% and 15% of the infiltrating lymphocytes, respectively.[24] Several of our patients received *H pylori* eradication therapy, and their LyGa was observed to regress. There may be a pathogenetic relationship between *H pylori* and LyGa. However, ~ 82% of the Japanese population is infected with *H pylori*.[25] Moreover, even patients who did not undergo eradication therapy exhibited regression of LyGa. In terms of the relation of LyGa with adenocarcinoma, LyGa is more likely to be found in persons who have frequently

undergone gastroscopy because LyGa shows no gastric symptoms. Therefore, although these concomitant occurrences appear coincidental, further studies are required for a better understanding of LyGa and its relationship with adenocarcinoma.

Whether LyGa is monoclonal proliferation or not remains a matter of debate. Unlike B or T cells, NK cells do not undergo any specific gene rearrangement, rendering it difficult to determine whether the proliferation of EBV-free NK cells is monoclonal or not. Vega et al[23] indicated that the NK-cell proliferation in their study appeared polyclonal because of the heterogeneous expression of the immunoglobulin-like receptors CD158a, CD158b, and CD158e; nevertheless, they could not exclude the possibility of a low-grade neoplasm. Siu et al[26] reported that the p73 gene was methylated in 94% of the NK-cell malignancies and that other methylated genes included *hMLH1* (63%), *p16* (63%), *p15* (48%), and *RAR* β (47%). We analyzed the methylation status of several genes, including *p16*, *p73*, *DAPK*, *MGMT*, *CDH1*, and *hMLH1*, in 2 heterochronically biopsied specimens from case 3 to obtain evidence of monoclonality. No aberrant methylation, however, was found in the examined genes (data not shown). These results reconfirmed that LyGa is different from extranodal NK/T-cell lymphoma, but the results did not serve as evidence for the monoclonality of LyGa. Further investigation with a larger sample size is required to clarify this distinction. Cytogenetic analyses and studies involving the identification of genetic loss/gain (eg, studies involving single nucleotide polymorphism microarray analysis) or point mutations (eg, studies involving next-generation genome sequencing) may be helpful to clarify the biologic natures of LyGa, especially whether LyGa is monoclonal proliferation or not. Procurement of fresh materials for these studies is impeded by spontaneous regression of lesions after the index biopsy; the biopsy specimen is usually fixed in formalin and embedded in paraffin for routine pathologic diagnosis.

LyGa should be regarded as a distinctive clinicopathologic entity and be observed without treatment. However, if not well recognized, LyGa is probably to be histopathologically misdiagnosed as lymphoma. For example, Kikuchi-Fujimoto disease, a self-limiting disorder of unknown cause, is still often mistakenly diagnosed as lymphoma,[4] although > 30 years have passed since it was first described in 1972. If LyGa is misdiagnosed as NK/T-cell lymphoma, it might be treated with radical therapeutic procedures, including chemotherapy, radiotherapy, gastrectomy, and stem cell transplantation. In fact, 2 patients of the present series underwent gastrectomy. The remaining 8 patients did not receive any treatment because the staging procedures followed by the initial diagnosis showed that the lesions regressed spontaneously. For 1 patient, however, the first biopsy specimen diagnosed as lymphoma was suspected to have been mistakenly identified to the patient. Fortunately, LyGa shows highly conserved and characteristic features in terms of clinical presentation, morphology, and immunophenotype (immunohistochemistry for CD3, CD5, CD7, CD56, and cytotoxic molecule(s) and EBER in situ hybridization are required to diagnose LyGa). Therefore, as long as LyGa is recognized as a distinct disease concept, there is no scope of misdiagnosis as malignancy.

Acknowledgments

We thank Drs Hiroshi Takahashi, Toshio Kumasaka, Yukiko Itoh, Satoko Hatano, Keiko Yoshimura, Kazuya Kobori, and Takanori Kuwabara and the members of Ganken Ariake Lymphoma Study Group (GALSG) for their advice.

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

Authorship

Contribution: K.T. and K.O. conceived the study, collected and analyzed the data, and drafted the paper; M.Y., Y.T., K. Marutsuka, M.N., N.F., T.Y., H.N., F.A., K. Hoshi, K. Matsue, and K. Hatake contributed patient materials and analyzed the data; and S.I. and K.N. performed special studies and analyzed the data.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Kengo Takeuchi, Pathology Project for Molecular Targets, Cancer Institute, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto, Tokyo 135-8550, Japan; e-mail: kentakeuchi-tky@umin.net.

References

1. Swerdlow SH, Campo E, Harris NL, et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: IARC Press; 2008.
2. Kikuchi M. Lymphadenitis showing focal reticulum cell hyperplasia with nuclear debris and phagocytosis. *Nippon Ketsueki Gakkai Zasshi.* 1972;35:379-380.
3. Fujimoto Y, Kozima Y, Yamaguchi K. Cervical subacute necrotizing lymphadenitis: a new clinicopathologic entity. *Naika.* 1972;20:920-927.
4. Menasce LP, Banerjee SS, Edmondson D, Harris M. Histiocytic necrotizing lymphadenitis (Kikuchi-Fujimoto disease): continuing diagnostic difficulties. *Histopathology.* 1998;33(3):248-254.
5. Kamel OW, van de Rijn M, Weiss LM, et al. Brief report: reversible lymphomas associated with Epstein-Barr virus occurring during methotrexate therapy for rheumatoid arthritis and dermatomyositis. *N Engl J Med.* 1993;328(18):1317-1321.
6. Oshimi K. Progress in understanding and managing natural killer-cell malignancies. *Br J Haematol.* 2007;139(4):532-544.
7. Suzuki R, Takeuchi K, Ohshima K, Nakamura S. Extranodal NK/T-cell lymphoma: diagnosis and treatment cues. *Hematol Oncol.* 2008;26(2):66-72.
8. The World Health Organization classification of malignant lymphomas in Japan: incidence of recently recognized entities. Lymphoma Study Group of Japanese Pathologists. *Pathol Int.* 2000;50(9):696-702.
9. Au WY, Ma SY, Chim CS, et al. Clinicopathologic features and treatment outcome of mature T-cell and natural killer-cell lymphomas diagnosed according to the World Health Organization classification scheme: a single center experience of 10 years. *Ann Oncol.* 2005;16(2):206-214.
10. Ko YH, Kim CW, Park CS, et al. REAL classification of malignant lymphomas in the Republic of Korea: incidence of recently recognized entities and changes in clinicopathologic features. Hematolymphoreticular Study Group of the Korean Society of Pathologists. Revised European-American lymphoma. *Cancer.* 1998;83(4):806-812.
11. Chen CY, Yao M, Tang JL, et al. Chromosomal abnormalities of 200 Chinese patients with non-Hodgkin's lymphoma in Taiwan: with special reference to T-cell lymphoma. *Ann Oncol.* 2004;15(7):1091-1096.
12. Harabuchi Y, Yamanaka N, Kataura A, et al. Epstein-Barr virus in nasal T-cell lymphomas in patients with lethal midline granuloma. *Lancet.* 1990;335(8682):128-130.
13. Jaffe ES, Chan JK, Su IJ, et al. Report of the Workshop on Nasal and Related Extranodal Angiocentric T/Natural Killer Cell Lymphomas. Definitions, differential diagnosis, and epidemiology. *Am J Surg Pathol.* 1996;20(1):103-111.
14. Aviles A, Diaz NR, Neri N, Cleto S, Talavera A. Angiocentric nasal T/natural killer cell lymphoma: a single centre study of prognostic factors in 108 patients. *Clin Lab Haematol.* 2000;22(4):215-220.
15. Ribrag V, El Hajj M, Janot F, et al. Early locoregional high-dose radiotherapy is associated with long-term disease control in localized primary angiocentric lymphoma of the nose and nasopharynx. *Leukemia.* 2001;15(7):1123-1126.
16. Shikama N, Ikeda H, Nakamura S, et al. Localized aggressive non-Hodgkin's lymphoma of the nasal cavity: a survey by the Japan Lymphoma Radiation Therapy Group. *Int J Radiat Oncol Biol Phys.* 2001;51(5):1228-1233.
17. Zhang YC, Sha Z, Yu JB, et al. Gastric involvement of extranodal NK/T-cell lymphoma, nasal

type: a report of 3 cases with literature review. *Int J Surg Pathol.* 2008;16(4):450-454.

18. Kim JH, Lee JH, Lee J, et al. Primary NK-/T-cell lymphoma of the gastrointestinal tract: clinical characteristics and endoscopic findings. *Endoscopy.* 2007;39(2):156-160.
19. Ko YH, Cho EY, Kim JE, et al. NK and NK-like T-cell lymphoma in extranasal sites: a comparative clinicopathological study according to site and EBV status. *Histopathology.* 2004;44(5):480-489.
20. Sasaki M, Matsue K, Takeuchi M, Mitome M, Hirose Y. Successful treatment of disseminated nasal NK/T-cell lymphoma using double autologous peripheral blood stem cell transplantation. *Int J Hematol.* 2000;71(1):75-78.
21. Chan JK, Tsang WY, Lau WH, et al. Aggressive T/natural killer cell lymphoma presenting as testicular tumor. *Cancer.* 1996;77(6):1198-1205.
22. Zettl A, deLeeuw R, Haralambieva E, Mueller-Hermelink HK. Enteropathy-type T-cell lymphoma. *Am J Clin Pathol.* 2007;127(5):701-706.
23. Vega F, Chang CC, Schwartz MR, et al. Atypical NK-cell proliferation of the gastrointestinal tract in a patient with antigliadin antibodies but not celiac disease. *Am J Surg Pathol.* 2006;30(4):539-544.
24. Yun CH, Lundgren A, Azem J, et al. Natural killer cells and Helicobacter pylori infection: bacterial antigens and interleukin-12 act synergistically to induce gamma interferon production. *Infect Immun.* 2005;73(3):1482-1490.
25. Uemura N, Okamoto S, Yamamoto S, et al. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med.* 2001;345(11):784-789.
26. Siu LL, Chan JK, Wong KF, Kwong YL. Specific patterns of gene methylation in natural killer cell lymphomas: p73 is consistently involved. *Am J Pathol.* 2002;160(1):59-66.