histopathological findings suggest microthrombosis or infarction in the lesions, followed by induction of a host immune response (Fig. 2). Discontinuous use of vorinostat 200 mg/day, together with aspirin 100 mg/day was well-tolerated. Although vorinostat was tolerated at a dose of 400 mg/day in the phase I study,[17] the dose is reduced to 200–300 mg/day in many cases because of gastrointestinal symptoms and general fatigue.

It is intriguing to note that even in the same patients, some lesions responded to vorinostat, while others would not. We believe that a combination of skin-directed therapy with phototherapy, radiotherapy or chemotherapy should be considered for vorinostat-resistant lesions.

Biological response modifier: Interferon-γ for MF/SS

Interferon (IFN)-α and IFN-γ were recommended as first-line therapy, in combination with skin-directed treatments, for patients with stages IIB and III MF/SS, and a second-line therapy for stages IA, IB to IIIB patients (Tables 1,2).[9] MF/SS are regarded as T-helper (Th)2-skewed disease characterized by eosinophilia, elevated serum levels of immunoglobulin E, interleukin (IL)-4 and IL-5,[18] and the expression of Th2-related CC chemokine ligand (CCL)11, CCL17 and CCL26.[19] Furthermore, our case with SS showed aggravation of the illness associated with a Th2-skewed immune reaction on staphylococcal infections, and improved with a Th1 shift on *Mycobacterium avium* infection.[20] It is, therefore, convincing that biological response modifiers such as IFN-α and IFN-γ have previously been used for treatments of MF/SS, in combination with skin-directed therapy.[21] Unfortunately, previously marketed IFN-γ products have not been available for treatment of MF/SS and ATLL since 2010. We conducted a multicenter, open-label, non-randomized, single-arm phase II study to evaluate the efficacy and safety of IFN-γ (Imunomax-γ; Shionogi, Osaka, Japan) for patients with stage IA to IIIA MF.[22] Of 15 patients who received 2 million Japan reference units of IFN-γ, once a day, over 5 days for 4 weeks, an objective response was observed in 11 patients (73.3%) by the response criteria of Ishihara *et al.*,[23] and in nine patients (60.0%) by the modified severity weighted assessment tool.[24] Influenza-like symptoms occurred in all patients, but such adverse reactions were tolerable. One patient died of aggravation of MF 50 days after the initiation of the study protocol. The phase II study proved that IFN-γ therapy was effective and tolerable in the management of patients with MF.

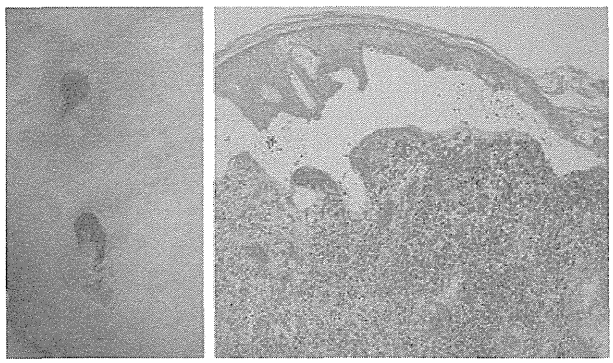

Figure 2. Acute tumor necrosis associated with thrombosis after intake of vorinostat.

Bexarotene for MF/SS

Bexarotene is a synthetic retinoid analog named "rexinoid" that specifically activates retinoid X receptors. Bexarotene has been approved in the USA and Europe for the treatment of refractory CTCL. Bexarotene therapy is generally initiated at the lower dose of 150 mg/m^2 per day for 2–4 weeks, then titrated up to an optimal full dose of 300 mg/m^2 per day.[25–29] Topical bexarotene therapy has also been reported as having benefits for refractory or persistent early-stage MF.[30] Overall response rates were 54% in early stage MF (stages IA–IIA) and 45% in advanced stage MF (stage IIB–IVB).[25,26] Prescribing physicians must monitor hyperglycemia, especially triglyceridemia and central hypothyroidism due to the decreased secretion of thyroid-stimulating hormone. Bexarotene is contraindicated in pregnancy. Clinical studies of oral bexarotene for management of CTCL in Japan are close to being completed.

Gemcitabine for MF/SS

Gemcitabine (2′,2′-difluorodeoxycytidine) is a pyrimidine analog which has been used for treatment of leukemias and lymphomas including CTCL.[31–34] Overall response rates to gemcitabine have ranged 62.5–75% for CTCL patients, depending on the administration doses, clinical stages of patients and pretreatments. Adverse reactions have included bone marrow suppression, hemolytic uremic syndrome, pulmonary embolism, hepatic damage, mucositis, infections, cardiac failure, influenza-like symptoms and skin rashes.

Combination therapy for MF/SS

Various combination therapies are usually required for management of MF/SS because various types and T stages of skin lesions are intermingled in the same individuals. The usual combination may include retinoids plus phototherapy, retinoids plus IFN or phototherapy plus vorinostat (Table 1). One prospective, randomized clinical trial showed that IFN-α plus psoralen plus ultraviolet A therapy (PUVA) was superior to IFN-α plus acitretin in achieving complete remission in CTCL stages I and II.[10] Another report has proved the beneficial effects of PUVA plus IFN-α in achieving high remission rates and prolonging progression-free survival when compared with PUVA alone.[35] Combinations of conventional skin-directed treatments with vorinostat and gemcitabine have been under investigation.

CUTANEOUS T/NK-CELL LYMPHOMA OTHER THAN MF/SS (NON-MF/SS)

Non-MF/SS cutaneous lymphomas are classified into two broad categories: (i) relatively aggressive lymphomas with poor prognosis (aggressive group); and (ii) indolent lymphomas with favorable prognosis (indolent group). The former group includes primary cutaneous CD8-positive aggressive epidermotropic

cytotoxic T-cell lymphoma, primary cutaneous γδ T-cell lymphoma, and peripheral T-cell lymphoma, not otherwise specified. It is, however, difficult to predict the prognosis of individual patients by cytological and immunophenotypic findings, without observation periods. For patients without general symptoms or notable laboratory test findings, skin-directed therapies used for MF/SS may be chosen as a first-line treatment.

An algorithm has been prepared for patients with indolent cutaneous lymphomas, which includes primary cutaneous anaplastic large cell lymphoma (pcALCL), subcutaneous panniculitis-like T-cell lymphoma and primary cutaneous CD4-positive small/medium T-cell lymphoma.[9] Because patients with pcALCL may take a progressive clinical course with regional lymph node involvement or disseminated skin lesions, polychemotherapy is sometimes required. Chimeric and humanized anti-CD30 monoclonal antibodies such as brentuximab (SGN-30) and MDX-060, both showed low overall response rates.[36,37] Brentuximab vedotin (SGN-35) is a conjugate of antitubulin agent monomethyl auristatin E and CD30-specific monoclonal antibody, which was designed to enhance antitumor activity. In 2011, brentuximab vedotin was approved for treatment of relapsed CD30-positive lymphomas, with objective responses in 17 of 45 patients with refractory disease.[38]

ATLL

Adult T-cell leukemia/lymphoma is a peripheral T-cell malignancy caused by human T-cell leukemia virus type 1 (HTLV-1) and occurs in certain areas where HTLV-1 infections are endemic, including Asia. Three major infection routes have been proven: (i) blood transfusion from HTLV-1 carriers; (ii) breast feeding; and (iii) sexual transmission, mainly from male to female. A sero-epidemiological survey by Tajima *et al.*[39] demonstrated that HTLV-1 infections are prevalent in Japanese, native Andeans, Iranians, Central Africans and those of African descent in the Caribbean Basin and South America. It is noteworthy that extremely low incidences of seropositivity and occurrence of ATLL were found in Korea and Eastern China, neighboring countries of Japan.

Shimoyama[40] classified ATLL into four subgroups based on hematological findings, blood chemistry results and organ involvements: acute, chronic, lymphomatous and smoldering types. More than 50% of ATLL patients present with cutaneous lesions including disseminated papules, nodules and tumors. Scaly erythemic plaques and erythroderma indistinguishable from those of MF/SS occur in some patients. Approximately 5% of HTLV-1 carriers may develop ATLL or HTLV-1-associated disorders in 50 years. In other words, the remaining 95% of HTLV-1 carriers do not experience any HTLV-1-related disorders throughout their lives even though they continue to harbor HTLV-1-infected T-cells.

Initial treatment for ATLL

Treatments of choice for ATLL should be determined by the subtypes and patients' conditions. For patients with ATLL lesions limited to the skin, PUVA, radiotherapy, oral retinoids, monochemotherapy using etoposide or a combination of these may be useful although beneficial effects on the prognosis of patients have not been confirmed.[9]

Recommended polychemotherapy for acute and lymphomatous types includes vincristine, cyclophosphamide, doxorubicin and prednisolone (VCAP), doxorubicin, ranimustine and prednisolone (AMP), and vincristine, etoposide, carboplatin and prednisolone (VEMP) regimens. The VCAP-AMP-VEMP (modified LSG15) regimen may be superior to biweekly CHOP, but the median survival time of 13 months still compares unfavorably to other T-cell malignancies.[41] A combination treatment with IFN-α and zidovudine may result in favorable response rates, particularly in acute, chronic and smoldering types of ATL.[41] Allo-hematopoietic stem cell transplant (HSCT) is a possible option for young patients with aggressive ATLL, but it remains to be answered which protocol of allo-HSCT is suitable for ATLL. Biologics have been developed for treatment of ATLL because of the unique immunophenotypic expression of CD2, CD25, CD52 and the Th2 type chemokine receptor, CCR4.

Mogamulizumab for refractory ATLL

Mogamulizumab is an humanized anti-CCR4 monoclonal antibody with a defucosylated Fc region, which markedly enhances antibody-dependent cellular cytotoxicity. CCR4 is known to be expressed on regulatory T cells, Th2 cells and ATLL cells. Therefore, a multicenter phase II study was conducted to assess the efficacy, pharmacokinetic profile and safety in patients with relapsed CCR4-positive aggressive ATLL.[42] The patients received eight weekly i.v. infusions of mogamulizumab 1.0 mg/kg. Objective responses were observed in 13 (50%) of 26 patients (95% confidence interval, 30–70%) (Fig. 3). The most common adverse events were infusion reactions and skin rashes. Duvic *et al.*[43] recently reported a phase I/II study of mogamulizumab for refractory CTCL with or without CCR4 expression, showing an overall response rate of 39%.

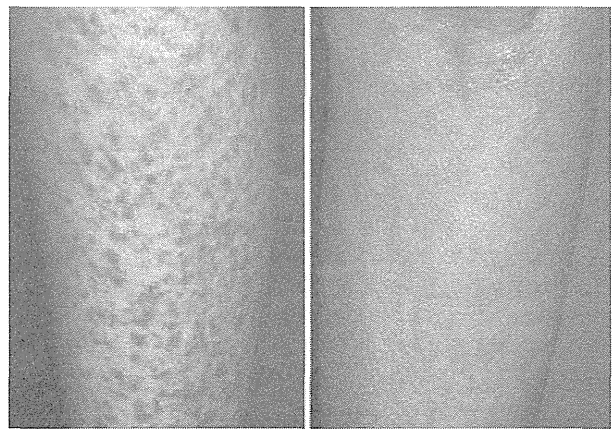

Figure 3. A patient with adult T-cell leukemia/lymphoma before and after mogamulizumab treatment.

ENKL

In addition to a prototype of Epstein–Barr virus (EBV)-associated NK/T-cell lymphomas, ENKL, hydroa vacciniforme (HV)-like lymphoma has been listed in the World Health Organization (WHO) classification 2008 (Fig. 4). As reported by Lee and Ko,[44] both ENKL and HV-like lymphoma have been reported in Mexico, Peru and Asia, including Japan. The vast majority of ENKL cases are caused by EBV-infected NK cells, whereas HV-like lymphoma is a form of EBV-associated T-cell lymphoma. HV-like lymphomas occur most frequently in children and adolescents, and are often accompanied by hypersensitivity to mosquito bites and hemophagocytic syndrome (HPS). The prognosis of patients with classical HV is usually favorable, but one-third of patients with systemic HV (synonymous for HV-like lymphoma) died of HPS and multi-organ failure 10 years after onset (Miyake T, Yamamoto T, Hirai Y, Otsuka M, Hamada T, Morizane S, Iwatsuki K, unpubl. data). Univariate analysis revealed two poor prognostic indicators in such cases: (i) onset age over 9 years; and (ii) the expression of an EBV reactivation signal, BZLF1, in the skin lesions.

Radiation therapy with a simultaneous or subsequent DeVIC regimen (dexamethasone, VP16, ifosfamide, carboplatin) is recommended for localized lesions, and the SMILE regimen (dexamethasone, methotrexate, ifosfamide, L-asparaginase and etoposide) for generalized lesions of ENKL.[45] For HV-like lymphoma, polychemotherapy followed by HSCT has been chosen in many cases,[46] but HSCT-related complications and death occasionally occur.

CUTANEOUS B-CELL LYMPHOMA

The WHO 2008 classification of hematopoietic malignancies has listed the nomenclature of cutaneous B-cell lymphomas as follows: extranodular marginal zone B-cell lymphoma (cMZL); primary cutaneous follicle center cell lymphoma (PCFCL), primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, leg type); PCLBCL, not otherwise specified; and intravascular large B-cell lymphoma (IVL). Disease type is an important prognostic factor for cutaneous B-cell lymphoma. Both PCFCL and MALT-type lymphomas are indolent-type lymphomas with a favorable prognosis, while prognosis is poor in PCLBCL and IVL. In 2008, the European Organization for Research and Treatment of Cancer and International Society for Cutaneous Lymphomas released guidelines for the treatments of cutaneous B-cell lymphoma, based on previous reports.[47] Most of the reported treatment methods for topical therapy involved radiation and/or surgical resection. Radiotherapy or surgical resection is recommended for diseases in the indolent group (cMZL and PCFCL).

Most of the methods for systemic therapy involve chemotherapy and the administration of rituximab.[48] Rituximab may be useful for the treatment of diseases in the indolent group (cMZL and PCFCL), particularly in cases of multiple lesions. One should consider that $CD20^{-}$ $CD138^{+}$ plasmacytoid neoplastic cells are resistant to rituximab monotherapy. Combination chemotherapy may be considered for diseases in the indolent group that are refractory to other treatment regimens and for advanced extracutaneous disease. Combination chemotherapy, and particularly the concomitant use of rituximab, is recommended for PCLBCL, leg type, and for IVL, but rituximab monotherapy is also possible for the treatment of PCLBCL in cases where combination therapy may be poorly tolerated, such as in the elderly and in patients with severe complications.

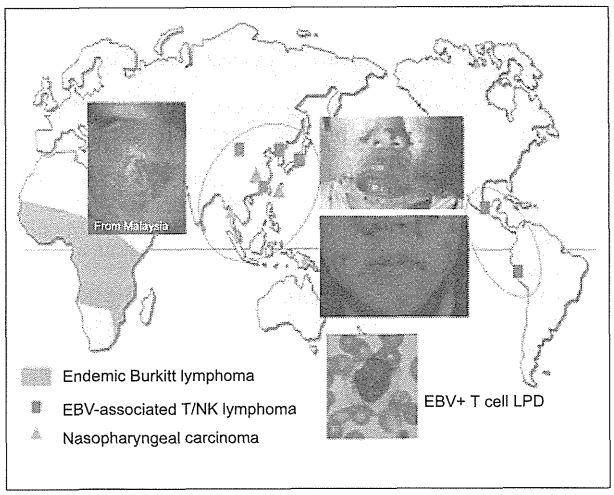

Figure 4. Hydroa vacciniforme-like lymphoma (synonymous with systemic hydroa vacciniforme) in Asia.

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REFERENCES

1 Hamada T, Iwatsuki K. Cutaneous lymphomas in Japan: a nationwide study of 1,733 patients. *J Dermatol* 2014; **41**: 3–10.
2 Lee MY, Tan TD, Feng AC *et al.* Clinicopathological analysis of malignant lymphoma in Taiwan, defined according to the World Health Organization classification. *Haematologica* 2005; **90**: 1703–1705.
3 Lymphoma Study Group of Japanese Pathologists. The World Health Organization classification of malignant lymphomas in Japan: incidence of recently recognized entities. *Pathol Int* 2000; **50**: 696–702.

4 Ko YH, Kim H, Oh Y *et al.* EBV-associated T and NK cell lymphoproliferative disorders: consensus report of the 4th Asian Hematopathology Workshop. *J Hematopathol* 2012; **5**: 319–324.
5 Kim JM, Ko YH, Lee SS *et al.* WHO Classification of malignant lymphomas in Korea: report of the third nationwide study. *Korean J Pathol* 2011; **45**: 254–260.
6 Yang QP, Zhang WY, Yu JB *et al.* Subtype distribution of lymphomas in southwest China: analysis of 6382 cases using WHO classification in a single institution. *Diagn Pathol* 2011; **6**: 77.
7 Iwatsuki K, Kawai K, Ohtsuka M *et al.* Guidelines for the management of cutaneous malignant tumors II: cutaneous lymphomas. *Jap J Dermatol* 2009; **119**: 1189–1211.
8 Sugaya M. Guidelines for the management of cutaneous lymphomas (2011). *Jap J Dermatol* 2012; **122**: 1513–1531.
9 Sugaya M, Hamada T, Kawai K *et al.* Guidelines for the management of cutaneous lymphomas (2011): a consensus statement by the Japanese Skin Cancer Society – Lymphoma Study Group. *J Dermatol* 2013; **40**: 2–14.
10 Stadler R, Otte HG, Luger T *et al.* Prospective randomized multicenter clinical trial on the use of interferon-2a plus acitretin versus interferon-2a plus PUVA in patients with cutaneous T-cell lymphoma stages I and II. *Blood* 1998; **92**: 3578–3581.
11 Stadler R, Kremer A, Luger T, Sterry W. Prospective, randomized, multicentre clinical trial on the use of interferon a 2a plus PUVA versus PUVA monotehrapy in patients with cutaneous T-cell lymphoma, stage I and II. *J Clin Oncol* 2006 ASCO Annual Meeting Proceedings 2006; **24**: 7541.
12 Kaye FJ, Bunn PA Jr, Steinberg SM *et al.* A randomized trial comparing combination electron-beam radiation and chemotherapy with topical therapy in the initial treatment of mycosis fungoides. *N Engl J Med* 1989; **321**: 1784–1790.
13 Child FJ, Mitchell TJ, Whittaker SJ *et al.* A randomized cross-over study to compare PUVA and extracorporeal photopheresis in the treatment of plaque stage (T2) mycosis fungoides. *Clin Exp Dermatol* 2004; **29**: 231–236.
14 Prince HM, Duvic M, Martin A *et al.* Phase III placebo-controlled trial of denileukin diftitox for patients with cutaneous T-cell lymphoma. *J Clin Oncol* 2010; **28**: 1870–1877.
15 Foss FM, Zinzari PL, Vose JM *et al.* Peripheral T-cell lymphoma. *Blood* 2011; **117**: 6756–6767.
16 Olsen EA, Kim YH, Kuzel TM *et al.* Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol* 2007; **25**: 3109–3115.
17 Wada H, Tsuboi R, Kato Y *et al.* Phase 1 and pharmacokinetic study of the oral histone deacetylase inhibitor vorinostat in Japanese patients with relapsed or refractory cutaneous T-cell lymphoma. *J Dermatol* 2012; **39**: 823–828.
18 Dummer R, Heald PW, Nestle FO *et al.* Sézary syndrome T-cell clones display T-helper 2 cytokines and express the accessory factor-1 (interferon-gamma receptor beta-chain). *Blood* 1996; **88**: 1383–1389.
19 Kakinuma T, Sugaya M, Kaneko F *et al.* Thymus and activation-regulated chemokine (TARC/CCL17) in mycosis fungoides: serum TARC levels reflect the disease activity of mycosis fungoides. *J Am Acad Dermatol* 2003; **48**: 23–30.
20 Yamada A, Yamasaki O, Asagoe K *et al.* Recovery from Sezary syndrome following *Mycobacterium avium* spondylitis. *Br J Dermatol* 2007; **157**: 1270–1271.
21 Whittaker SJ, Foss FM. Efficacy and tolerability of currently available therapies for the mycosis fungoides and Sezary syndrome variants of cutaneous T-cell lymphoma. *Cancer Treat Rev* 2007; **33**: 146–160.
22 Sugaya M, Tokura Y, Hamada T *et al.* Phase II study of intravenous interferon-g in Japanese patients with mycosis fungoides. *J Dermatol* 2014; **41**: 50–56.
23 Ishihara K. Late phase II clinical study of OH-6000 for adult T cell leukemia and lymphoma. *Skin Cancer* 1993; **8**: 352–367.
24 Olsen EA, Whittaker S, Kim YH *et al.* Clinical end points and response criteria in mycosis fungoides and Sézary syndrome: a consensus statement of the International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer. *J Clin Oncol* 2011; **29**: 2598–2607.
25 Duvic M, Martin AG, Kim Y *et al.* Phase 2 and 3 clinical trial of oral bexarotene (Targretin capsules) for the treatment of refractory or persistent early-stage cutaneous T-cell lymphoma. *Arch Dermatol* 2001; **137**: 581–593.
26 Duvic M, Hymes K, Heald P *et al.* Bexarotene is effective and safe for treatment of refractory advanced-stage cutaneous T-cell lymphoma: multinational phase II–III trial results. *J Clin Oncol* 2001; **19**: 2456–2471.
27 Gniadecki R, Assaf C, Bagot M *et al.* The optimal use of bexarotene in cutaneous T-cell lymphoma. *Br J Dermatol* 2007; **157**: 433–440.
28 Querfeld C, Rosen ST, Guitart J *et al.* Comparison of selective retinoic acid receptor- and retinoic X receptor-mediated efficacy, tolerance, and survival in cutaneous t-cell lymphoma. *J Am Acad Dermatol* 2004; **51**: 25–32.
29 Breneman D, Duvic M, Kuzel T *et al.* Phase 1 and 2 trial of bexarotene gel for skin-directed treatment of patients with cutaneous T-cell lymphoma. *Arch Dermatol* 2002; **138**: 325–332.
30 Heald P, Mehlmauer M, Martin AG *et al.* Topical bexarotene therapy for patients with refractory or persistent early-stage cutaneous T-cell lymphoma: results of the phase III clinical trial. *J Am Acad Dermatol* 2003; **49**: 801–815.
31 Jidar K, Ingen-Housz-Oro S, Beylot-Barry M *et al.* Gemcitabine treatment in cutaneous T-cell lymphoma: a multicenter study of 23 cases. *Br J Dermatol* 2009; **161**: 660–663.
32 Duvic M, Talpur R, Wen S *et al.* Phase II evaluation of gemcitabine monotherapy for cutaneous T-cell lymphoma. *Clin Lymphoma Myeloma* 2006; **7**: 51–58.
33 Zinzani PL, Baliva G, Magagnoli M *et al.* Gemcitabine treatment in pretreated cutaneous T-cell lymphoma: experience in 44 patients. *J Clin Oncol* 2000; **13**: 2603–2606.
34 Marchi E, Alinari L, Tani M *et al.* Gemcitabine as frontline treatment for cutaneous T-cell lymphoma: phase II study of 32 patients. *Cancer* 2005; **104**: 2437–2441.
35 Roenigk HH Jr, Kuzel TM, Skouteils AP *et al.* Photochemotherapy alone or combined with interferon-2a in the treatment of cutaneous T-cell lymphoma. *J Invest Dermatol* 1990; **95** (6 Suppl): 198S–205S.
36 Forero-Torres A, Leonard JP, Younes A *et al.* A phase II study of SGN-30 (anti-CD30 mAb) in Hodgkin lymphoma or systemic anaplastic large cell lymphomas. *Br J Haematol* 2009; **146**: 171–179.
37 Ansell SM, Horwitz SM, Engert A *et al.* Phase I/II study of an anti-CD30 monoclonal antibody (MDX-060) in Hodgkin's lymphoma and anaplastic large-cell lymphoma. *J Clin Oncol* 2007; **25**: 2764–2769.
38 Younges A, Barlett N, Leonard JP *et al.* Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. *N Engl J Med* 2010; **363**: 1812–1821.
39 Tajima K, Takezaki T. Ethnoepidemiologicay of ATL/HTLV in Mongoloids with special reference to Mongoloid dispersal. *Gann Monogr Cancer Res* 2003; **50**: 277–288.
40 Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukemia-lymphoma. A report from the Lymphoma Study Group (1984–87). *Br J Haematol* 1911; **79**: 428–437.
41 Tsukasaki K, Tobinai K. Clinical trials and treatment of ATL. *Leuk Res Treatment* 2012; **2012**: 101754.
42 Ishida T, Joh T, Uike N *et al.* Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter pahse II study. *J Clin Oncol* 2012; **30**: 837–842.
43 Duvic M, Pinter-Brown L, Foss FM *et al.* Results of a phase 1/2 study for KW-0761, a monoclonal antibody directed against CC chemokine receptor type 4 (CCR4), in CTCL patients. ASH Annual Meeting Abstracts 2010 116: abstract 962, Clincal trials. Gov: NCT00888927), Orlando, USA, 2010.
44 Park S, Ko YH. Epstein-Barr virus-associated T/Natural killer (NK) cell lymphoproliferative disorders. *J Dermatol* 2014; **41**: 29–39.

45 Yamaguchi M, Kwong YL, Kim WS *et al.* Phase II study of SMILE chemotherapy for newly diagnosed stage IV, relapsed, or refractory extranodal natural killer (NK)/T-cell lymphoma, nasal type: the NK-cell Tumor Study Group study. *J Clin Oncol* 2011; **33**: 4410–4416.
46 Kawa K, Sawada A, Sato M *et al.* Excellent outcome of allogeneic hematopoietic SCT with reduced-intensity conditioning for the treatment of chronic active EBV infection. *Bone Marrow Transplant* 2011; **46**: 77–83.
47 Sneff NJ, Noordijk EM, Kim YH *et al.* European Organization for Research and Treatment of Cancer and International. Society for Cutaneous Lymphoma consensus recommendations for the management of cutaneous B-cell lymphomas. *Blood* 2008; **112**: 1600–1609.
48 Suárez AL, Querfeld C, Horwitz S, Pulitzer M, Moskowitz A, Myskowski PL. Primary cutaneous B-cell lymphomas: part II. Therapy and future directions. *J Am Acad Dermatol* 2013; **69**: 343.e1–343.e11.

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ORIGINAL ARTICLE

Cutaneous lymphoma in Japan: A nationwide study of 1733 patients

Toshihisa HAMADA, Keiji IWATSUKI

Department of Dermatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

ABSTRACT

Types of cutaneous lymphoma (CL) and their incidences may vary among geographic areas or ethnic groups. The present study aimed to investigate the incidences of various CL in Japan, using epidemiological data from a nationwide registration system for CL. Between 2007 and 2011, 1733 new patients with CL were registered from over 600 dermatological institutes in Japan. The 1733 patients registered included 1485 (85.7%) patients with mature T- and natural killer (NK)-cell neoplasms, 224 (12.9%) with B-cell neoplasms and 24 (1.4%) with blastic plasmacytoid dendritic cell neoplasm. Mycosis fungoides (MF) is the most common CL subtype in the present study (750 patients, 43.3%). The proportion of MF patients with early-stage disease was 73%, similar to that of previous studies from other cohorts. The incidence rates of adult T-cell leukemia/lymphoma and extranodal NK/T-cell lymphoma, nasal type were 16.7% and 2.0%, respectively, which may account for the higher incidence of mature T- and NK-cell neoplasms in Japan, as compared with that in the USA and Europe. A male predominance was observed in most types of CL, except for several CL subtypes such as subcutaneous panniculitis-like T-cell lymphoma.

Key words: adult T-cell leukemia/lymphoma, cutaneous lymphoma, extranodal natural killer/T-cell lymphoma, mycosis fungoides, nasal type, subcutaneous panniculitis-like T-cell lymphoma.

INTRODUCTION

Cutaneous lymphomas (CL) are the second most common type of extranodal non-Hodgkin's lymphoma, after gastrointestinal lymphomas.[1] CL are defined as lymphomas with skin infiltration of neoplastic lymphocytic cells, without nodal or internal involvement at diagnosis. The World Health Organization (WHO) classification for tumors of hematological and lymphoid tissue, including CL, was published in 2008, through several consensus meetings, and is based on a combination of clinicopathological, phenotypic, genetic and molecular characteristics.[2] Mycosis fungoides (MF) is the most common type of CL. In 2007, a revised version of the MF/Sézary syndrome (MF/SS) staging system was published, thereafter, a tumor–node–metastasis (TNM) classification system was proposed for CL other than MF/SS.[3,4] Using the new criteria, clinical outcomes including survival data have recently been reported from the UK and Japan.[5,6] However, these studies analyzed clinical data from only a single medical center over 25- or 30-year periods.

Cutaneous lymphoma is a rare disease entity, and is difficult to study on a large scale. Thus, most epidemiological surveys on CL have been limited to case series reports, mainly of single medical centers.[7–12] Epidemiologic data of CL has not been fully evaluated to date. Entry of data into a comprehensive registry of CL is required in many parts of the world. To date, a few large-scale epidemiological studies on CL have been performed mainly in the USA and Europe.[13–15] The findings from the present study, including the incidence rates of CL, may be somewhat different from those studies. Indeed, the incidence pattern of CL has been reported to be different by country or ethnic group, like that of gross lymphoproliferative disorders. For example, adult T-cell leukemia/lymphoma (ATLL) is endemic in southwest Japan, especially on Kyushu Island.[16,17] However, it is very rare in the USA and Europe.[13–15]

In 2007, we established a nationwide registry system for Japanese CL, in cooperation with the Japanese Skin Cancer Society (JSCS) Lymphoma Study Group. The present registry covers the whole country, and is aimed at elucidating the distinct pattern of Japanese CL, mainly using the WHO classification and the revised version of MF/SS clinical staging.[2,3] In addition, the present registry can minimize the kind of selection bias resulting from single-center analysis because data from hundreds of institutions throughout Japan are included. Such analyses will be conducted over the whole area of Japan each year. Thus, this registry will facilitate further clinical study and basic research in the near future.

METHODS

We analyzed the incidence pattern of CL from 2007 to 2011. The present registry covers the entire nation and includes more

Correspondence: Toshihisa Hamada, M.D., Department of Dermatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan. Email: t-hamada@cc.okayama-u.ac.jp
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than 600 dermatological institutes throughout Japan, all of which have been approved as residency programs for board-certified dermatologists by the Japanese Dermatological Association (JDA) (Table 1). On average, a total of 628 institutes per year participated in the present study. In addition, the total number of the registered institutes of each prefecture is shown in Table 1. The diagnosis of CL was confirmed according to the WHO classification mentioned above.[2] Subjects were newly diagnosed patients with CL in each institute. Clinical data including age at diagnosis, sex, TNM classification, clinical stage, anatomical site of the primary lesion, nodal or extracutaneous involvement, and initial therapy were retrieved from the medical database of each medical institute. In the present study, unconventional sites such as the groin were excluded from the statistical analyses, because of their small number. Those data were submitted electronically without personal information to our data center once a year. This study was approved by the ethics board committee (the review board of the JDA).

Table 1. Distribution of dermatological institutes

	Dermatological institutes (2007–2011)		
	Total no.	No. per year	%
Prefecture	3140	628	100
Hokkaido	157	31	5.0
Aomori	32	6	1.0
Iwate	29	6	0.9
Miyagi	54	11	1.7
Akita	22	4	0.7
Yamagata	21	4	0.7
Fukushima	39	8	1.2
Ibaraki	52	10	1.7
Tochigi	32	6	1.0
Gunma	40	8	1.3
Saitama	133	27	4.2
Chiba	79	16	2.5
Tokyo	397	79	12.6
Kanagawa	227	45	7.2
Niigata	33	7	1.1
Yamanashi	17	3	0.5
Nagano	52	10	1.7
Toyama	44	9	1.4
Ishikawa	49	10	1.6
Fukui	27	5	0.9
Gifu	63	13	2.0
Shizuoka	124	25	3.9
Aichi	231	46	7.4
Mie	33	7	1.1
Shiga	53	11	1.7
Kyoto	76	15	2.4
Osaka	249	50	7.9
Hyogo	143	29	4.6
Nara	41	8	1.3
Wakayama	30	6	1.0
Tokushima	20	4	0.6
Kagawa	23	5	0.7
Ehime	15	3	0.5
Kochi	22	4	0.7
Tottori	10	2	0.3
Shimane	20	4	0.6
Okayama	46	9	1.5
Hiroshima	88	18	2.8
Yamaguchi	38	8	1.2
Fukuoka	110	22	3.5
Saga	20	4	0.6
Nagasaki	33	7	1.1
Kumamoto	41	8	1.3
Oita	24	5	0.8
Miyazaki	18	4	0.6
Kagoshima	17	3	0.5
Okinawa	16	3	0.5

The comprehensive classification of CL and hematopoietic neoplasms with marked affinity for the skin was presented by the European Organization for Research and Treatment of Cancer (EORTC) in 2005.[13] This framework of CL classification was essentially duplicated by the WHO classification, with several nominal or hierarchical differences.[2] The present registry has dealt with CL, shown in Table 2. Clinical stage and TNM classification of patients with MF/SS were identified using the International Society for Cutaneous Lymphomas (ISCL)/EORTC proposal in 2007 (which was modified in 2011).[3,18]

RESULTS

In total, 1733 patients with CL have been registered between 2007 and 2011 (Table 2). The patients ranged 1–100 years (median, 65) in age, and included 978 males and 751 females (M : F ratio, 1.30). Mature T-cell and natural killer (NK)-cell neoplasm was the most common type of CL, accounting for 1485 (85.7%) patients. Next in prevalence, 224 (12.9%) of 1733 patients had mature B-cell neoplasm. The remaining 24 (1.4%) patients had blastic plasmacytoid dendritic cell neoplasm (BPDN).

Mycosis fungoides was the most common subtype of mature T-cell and NK-cell neoplasms, comprising 50.5% of cases, followed by ATLL (290 patients, 19.5%), primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders (208 patients, 14.0%), and peripheral T-cell lymphoma, not otherwise specified (100 patients, 6.7%). Other subtypes of mature T-cell and NK-cell neoplasms included 34 (2.3%) subcutaneous panniculitis-like T-cell lymphoma (SPTCL), 34 (2.3%) extranodal NK/T-cell lymphoma, nasal type (ENKL) and 33 (2.2%) SS cases. The incidences of rare disease entities including primary cutaneous $CD4^+$ small/medium T-cell lymphoma, primary cutaneous $\gamma\delta$ T-cell lymphoma and primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma were 25 (1.7%), five (0.3%) and six (0.4%), respectively.

The most common mature B-cell neoplasm subtype was primary cutaneous diffuse large-cell lymphoma, leg type (95 patients, 42.4%), followed by extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) (72 patients, 32.1%), primary cutaneous follicle center lymphoma (pcFCL) (37 patients, 16.5%) and intravascular large B-cell lymphoma (IVLBCL) (20 patients, 8.9%).

Table 2. Characteristics of cutaneous lymphomas between 2007 and 2011

	Total		Neoplasm	Male	Female		Age at diagnosis (years)		
	n	%	category (%)	*n*	*n*	M : F	Median	Average ± SD	Range
Total	1733	100.0		978	751	1.30	65	63.1 ± 16.5	1–100
Mature T-cell and NK-cell neoplasms	1485	85.7	100.0	838	643	1.30	64	62.2 ± 16.4	5–100
Mycosis fungoides	750	43.3	50.5	438	310	1.41	62	60.6 ± 15.5	13–95
Sézary syndrome	33	1.9	2.2	26	7	3.71	68	67.6 ± 12.3	37–89
Primary cutaneous CD30$^+$ T-cell lymphoproliferative disorders	208	12.0	14.0	117	91	1.29	63	59.4 ± 20.4	6–97
Primary cutaneous anaplastic large-cell lymphoma	136	7.8	9.2	84	52	1.62	67.5	63.5 ± 19.1	12–97
Lymphomatoid papulosis	66	3.8	4.4	30	36	0.83	53.5	51.1 ± 20.4	6–84
Subcutaneous panniculitis-like T-cell lymphoma	34	2.0	2.3	12	22	0.55	55	54.5 ± 16.3	17–81
Peripheral T-cell lymphoma, NOS	100	5.8	6.7	51	47	1.09	68	65.5 ± 18.4	5–100
Primary cutaneous CD4$^+$ small/medium T-cell lymphoma*	25	1.4	1.7	11	14	0.79	65	61.5 ± 20.7	14–90
Primary cutaneous γδ T-cell lymphoma	5	0.3	0.3	2	3	–	–	–	–
Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic T-cell lymphoma*	6	0.3	0.4	1	5	–	–	–	–
Extranodal NK/T-cell lymphoma, nasal type	34	2.0	2.3	13	21	0.62	66	65.6 ± 15.4	31–94
Adult T-cell leukemia/lymphoma	290	16.7	19.5	167	123	1.36	68	67.5 ± 11.9	19–91
Mature B-cell neoplasms	224	12.9	100.0	120	104	1.15	70	68.3 ± 16.2	1–94
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue	72	4.2	32.1	36	36	1.00	63	63.4 ± 16.7	20–94
Primary cutaneous follicle center lymphoma	37	2.1	16.5	25	12	2.08	64	67.1 ± 14.7	26–88
Primary cutaneous diffuse large-cell lymphoma, leg type	95	5.5	42.4	45	50	0.90	77	72.5 ± 16.7	1–92
Intravascular large B-cell lymphoma	20	1.2	8.9	14	6	2.33	70	72.4 ± 15.7	53–85
Immature hematological neoplasms†									
Blastic plasmacytoid dendritic cell neoplasm	24	1.4	–	20	4	5.00	77.5	74.5 ± 11.5	34–86

*Provisional. †Immature hematological neoplasms include "acute myeloid leukemia and related precursor neoplasms" and "precursor lymphoid neoplasms". NK, natural killer; NOS, not otherwise specified; SD, standard deviation.

A male predominance was observed in all CL (M : F ratio, 1.30), with over twofold male predominance for BPDN (M : F ratio, 5.00), SS (M : F ratio, 3.71), IVLBCL (M : F ratio, 2.33) and pcFCL (M : F ratio, 2.08). In contrast, a female predominance was observed in SPTCL (M : F ratio, 0.55), followed by in ENKL (M : F ratio, 0.62), primary cutaneous CD4$^+$ small/medium T-cell lymphoma (M : F ratio, 0.79) and lymphomatoid papulosis (LyP) (M : F ratio, 0.83).

The median age at diagnosis was low in patients with LyP (53.5 years) and SPTCL (55 years), as compared with that of all CL (65 years). In contrast, the median age at diagnosis was high in patients with BPDN (77.5 years) and primary cutaneous diffuse large-cell lymphoma (pcDLBCL), leg type (77 years). In general, the patient's age was high in mature B-cell neoplasm, as compared with those in mature T-cell and NK-cell neoplasm (median ages of 70 and 64 years, respectively). In reference to age distribution, bimodal distributions of age at diagnosis were found in LyP (the fourth and the sixth decades) and SPTCL (the fifth and the seventh decades).

Clinical stage of MF/SS (Table 3)

In terms of clinical staging, the 744 MF/SS patients included 229 (29.6%) with stage IA, 303 (39.1%) stage IB, 33 (4.3%) stage IIA, 86 (11.1%) stage IIB, 57 (7.4%) stage IIIA, seven (0.9%) stage IIIB, 17 (2.2%) stage IVA1, 28 (3.6%) stage IVA2 and 14 (1.8%) stage IVB. In all, 565 patients (73%) had early-stage disease (stage I + IIA). The remaining 209 (27%) patients had the advanced-stage disease (stage IIB + III + IV). A male predominance was observed in stage IIIA (M : F ratio, 4.18), stage IIIB (M : F ratio, 2.50) and stage IVA1 (M : F ratio, 2.40). In contrast, a female predominance was observed in stage IVA2 (M : F ratio, 0.75). The median ages at diagnosis were 61–62 years in stage IA to IIB and stage IIIB, and 64–70 years in stage IIIA and IV.

Anatomical site of the primary skin lesion (Fig. 1)

The skin lesion sites of primary cutaneous anaplastic large-cell lymphoma were distributed approximately evenly. The most commonly affected sites were the lower extremities in SPTCL

Table 3. Characteristics of clinical staging of MF/SS (2007–2011)

Clinical stage*	Total		Male	Female		Age at diagnosis (years)
	n	%	*n*	*n*	M/F	Median (range)
Total	774	100.0	459	314	1.46	62 (13–95)
IA	229	29.6	128	100	1.28	62 (16–92)
IB	303	39.1	177	126	1.40	62 (13–93)
IIA	33	4.3	19	14	1.36	61 (20–93)
IIB	86	11.1	51	35	1.46	61 (23–89)
IIIA	57	7.4	46	11	4.18	70 (33–95)
IIIB	7	0.9	5	2	2.50	61 (41–85)
IVA1	17	2.2	12	5	2.40	69 (37–83)
IVA2	28	3.6	12	16	0.75	64.5 (35–88)
IVB	14	1.8	9	5	1.80	64 (28–80)

*Clinical stage was identified using the International Society for Cutaneous Lymphomas/European Organization for Research and Treatment of Cancer proposal in 2007. MF, mycosis fungoides; SS, Sézary syndrome.

(62%) and the head/neck or the trunk in primary cutaneous $CD4^+$ small/medium T-cell lymphoma (52%). In pcFCL and MALT, the head and neck were commonly affected (84% and 56%, respectively). In contrast, the lower extremities were the most commonly affected sites in DLBCL, leg type (45%). BPDN preferentially arose on the trunk (83%).

DISCUSSION

In the present study, we aimed to reveal the distinct characteristics of the Japanese pattern of CL. After the initiation of the annual registry in 2007, 1733 newly diagnosed patients with CL have been registered from over 600 dermatological institutes throughout Japan. The present registry is not a "population-based" study in a precise sense. However, the data presented herein are believed to be representative of the Japanese CL. A possible limitation of the study includes uncertainty about the accuracy of the diagnostic procedure in each institute with lack of central pathology review. However, we believe this may not be a matter of great importance, because all enrolled institutes have residency programs for dermatologists to become board-certified by the JDA. By the present registry system, trends of overall incidence and disease distribution of CL in Japan will be evaluated continually.

We showed that the majority (85.7%) of CL cases were mature T-cell and NK-cell neoplasms, more or less similar to findings in previous studies from Japan and elsewhere.[7–15] However, in detail, the incidence rate of mature T-cell and NK-cell neoplasm in the present study was 8.7–14.4%, higher than in those of the USA and Europe. In contrast, the incidence of mature B-cell neoplasm (12.9%) was much lower: 10.1–15.6% lower than in the west (Table 4). It is noteworthy that the incidence rate of MALT in the present study was lower than in those of the USA and Europe. Occasionally, the distinction between B-cell pseudolymphoma and MALT can be very difficult in some patients.[19] Thus, one of the possible causes may include the diagnostic difficulty of MALT. The overall incidence pattern of CL in the present study was similar to that in previous studies from single centers of Japan and Korea (Table 4).[8,12] As compared with the incidence of CL in other countries or regions, MF/SS occurred at a similar frequency (45.2%) in Japan, while the incidence rates of ATLL and ENKL were observed to be 16.7% and 2.3%, respectively. The incidence rate of ENKL was higher than those of the USA and Europe, and lower than those of Korea and Taiwan.[11,12,20]

Adult T-cell leukemia/lymphoma is a distinct hematological neoplasm caused by the human T-cell lymphotropic virus type 1 (HTLV-1)-infected malignant $CD4^+$ T cells.[21–23] The endemic areas of ATLL include high-prevalence regions of HTLV-1, such as southwest Japan, various Caribbean countries, South America and Central Africa.[24–27] ATLL shows various clinical and prognostic features, and is classified into four categories according to the Shimoyama classification: acute, lymphoma, chronic and smoldering subtypes.[28] Cutaneous lesions are frequently observed in patients with ATLL, accounting for more than 50%.[21,29] Moreover, many types of ATLL-associated eruption have been reported to date.[30–32] The present study showed high prevalence of ATLL in Japan compared with other countries or regions including Korea (Table 4).

Extranodal NK/T-cell lymphoma, nasal type, is characterized by pleomorphic cell infiltration with NK-cell phenotype, which ordinarily demonstrates positivity to Epstein–Barr virus-encoded early small RNA by *in situ* hybridization.[2] Typically, pathological features include vascular damage and tissue necrosis by angiocentric infiltration of tumor cells. Frequently, ENKL affects the upper aerodigestive tract, followed by skin, soft tissue, the gastrointestinal tract and testes.[2] It is more prevalent in East Asia, Central America and South America than in Europe and the

Figure 1. The anatomical distribution sites of the primary skin lesion are shown in the graphic representation. (a) Primary cutaneous anaplastic large-cell lymphoma (pcALCL), (b) subcutaneous panniculitis-like T-cell lymphoma (SPTCL), (c) primary cutaneous $CD4^+$ small/medium T-cell lymphoma, (d) primary cutaneous follicle center lymphoma (pcFCL), (e) extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT), (f) primary cutaneous diffuse large-cell lymphoma, leg type (pcDLBCL, leg type) and (g) blastic plasmacytoid dendritic cell neoplasm (BPDN).

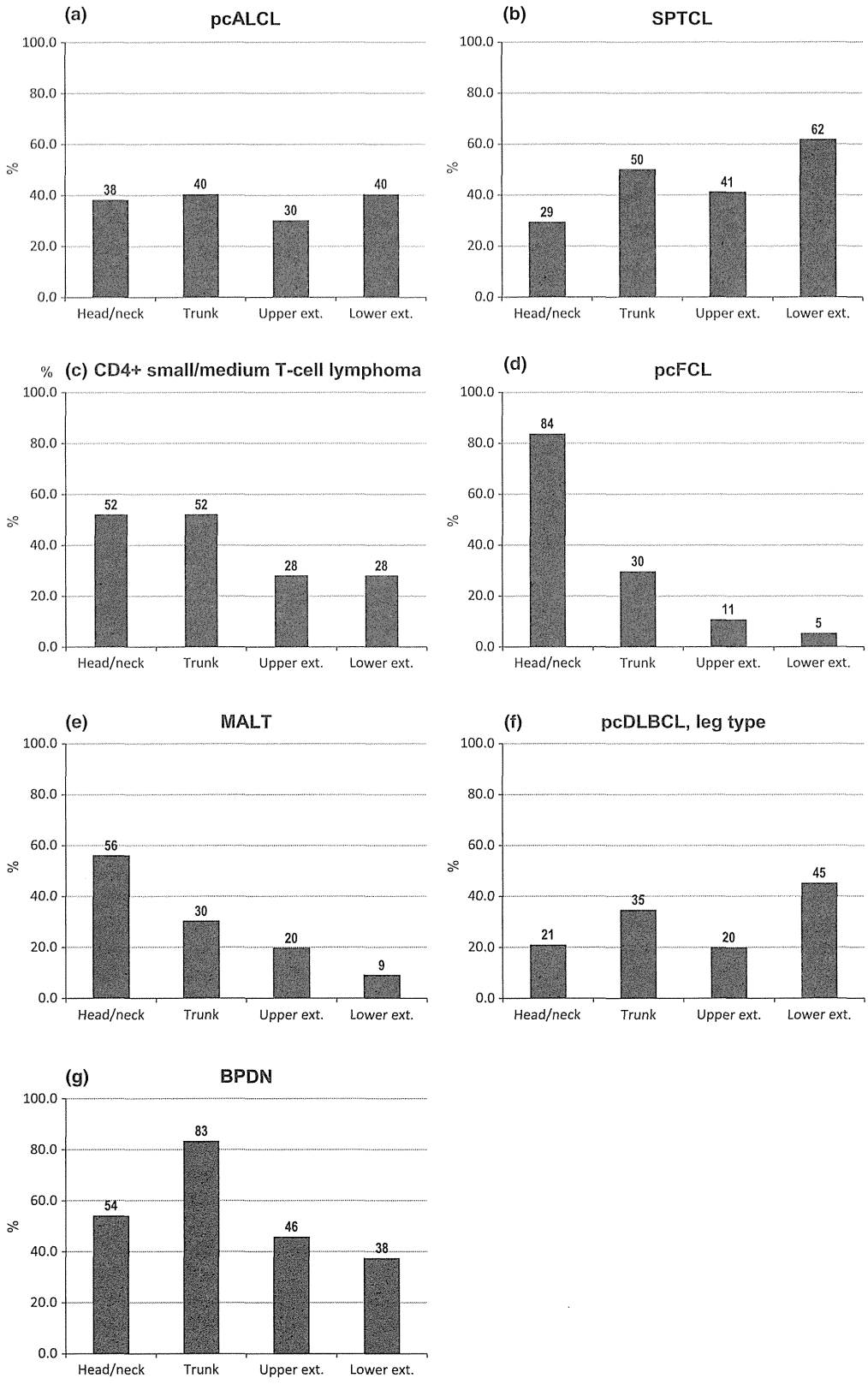

Table 4. Incidence patterns of cutaneous lymphomas from the present study and other cohorts

	Based on large-scale database			Survey from single medical centers			
Study group/registry or nation	JSCS	SEER16[15]	DACLG[13]	Switzerland[9]	France[7]	Korea[12]	Japan[8]
Total no.	1733	3884	1905	263	203	164	133
Surveillance period, year	5	5	17	20	7	16	14
Mature T-cell and NK-cell neoplasms	85.7	71.3	77	72	75.9	79.2	79.7
MF	43.3	38.3	47	43	43.3	14	41.4
SS	1.9	0.8	3	11	7.9	0.6	0.8
Primary cutaneous $CD30^+$ T-cell lymphoproliferative disorders	12.0	10.2		13			12.8
Primary cutaneous anaplastic large-cell lymphoma	7.8		8	8	3.5	14	6.8
Lymphomatoid papulosis	3.8		12	5	7.4	5.5	6.0
Subcutaneous panniculitis-like T-cell lymphoma	2.0	0.6	1		1	6.7	2.3
Peripheral T-cell lymphoma, NOS	5.8	20.8	2	2	1	4.9	3.8
Primary cutaneous $CD4^+$ small/medium T-cell lymphoma*	1.4		2	3	3	3	0.8
Primary cutaneous γδ T-cell lymphoma	0.3		<1		0.5	4.9	
Primary cutaneous $CD8^+$ aggressive epidermotropic cytotoxic T-cell lymphoma*	0.4				0.5		
Extranodal NK/T-cell lymphoma, nasal type	2.3	0.3	<1	<1	0	20.7	3.8
Adult T-cell leukemia/lymphoma	16.7	0.1				0.6	9.8
Mature B-cell neoplasms	12.9	28.5	23	28	24.1	16.5	18.0
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue	4.2	7.1	7	14	4.9	8.5	5.3
Primary cutaneous follicle center lymphoma	2.1	8.5	11	8	17.7	0	0.0
Primary cutaneous diffuse large-cell lymphoma, leg type	5.5	2.6	4	4	1	1.2	
Intravascular large B-cell lymphoma	1.2		<1		0.5	1.2	0.8
Immature hematological neoplasms†	1.4	0.3					
Blastic plasmacytoid dendritic cell neoplasm	1.4	0.2					0.8

*Provisional. †Immature hematological neoplasms include "acute myeloid leukemia and related precursor neoplasms" and "precursor lymphoid neoplasms". JSCS, Japanese Skin Cancer Society; MF, mycosis fungoides; NK, natural killer; NOS, not otherwise specified; SD, standard deviation; SS, Sézary syndrome.

USA.[13,33–35] Also, the incidence of ENKL in CL was reported to be significantly higher in Korea (15% and 20.7%) than in Europe and the USA.[12,20] In three single-institution studies from Japan, the incidence rate of ENKL has ranged 3.8–8.8%.[8,36,37] In the present study, the incidence was somewhat lower (2.3%) than these previous studies. The difference may reflect the kind of selection bias specific to single-institution studies. Our results suggest that the high incidence rates of mature T-cell and NK-cell neoplasm are associated with the prevalence of ATLL in Japan, unlike that of ENKL in Korea.

Mycosis fungoides is the most common CL subtype in the present study as well as in almost all counties or ethnicities. In the past, staging of MF/SS was performed according to the previously proposed staging system.[38,39] Prior to the establishment of the new staging system, several clinical studies had been conducted on relatively large cohorts of cutaneous T-cell lymphoma for such a rare disease entity.[40–42] In 2007, the revised staging system for MF/SS was released by the ISCL/EORTC (which was in turn modified in 2011).[3,18] This system adopted a newly proposed classification of tumor–node–metastasis–blood rating. Since then, two clinical studies of MF/SS have been conducted in the UK and Japan.[5,6] In the present study, the proportion of patients with early stage (IA to IIA) was 73%, similar to that of the previous studies (70.7% and 78%) (Table 5). Stage IB accounted for 39.1% of the total MF/SS in the present study, making it the most prevalent clinical stage. This finding is similar to the results of previous studies (38.8% and 38%) (Table 5).[5,6] In addition, a predominance of males among MF/SS patients was shown in the present study, as in previous reports.[5,6,8,9,12–15,20,40–42] Notably, a male predominance was observed in erythrodermic MF or SS, with over twofold male predominance for stage IIIA (M : F ratio, 4.18), stage IIIB (M : F ratio, 2.50) and stage IVA1 (M : F ratio, 2.40).

We evaluated the distinct anatomical distributions of the skin lesions in patients with several types of CL. In patients with pcFCL and MALT, the head and neck were the most commonly affected sites, as in previous reports from the USA and Asia.[15,43] By contrast, in Europe, the trunk was the most commonly affected site of pcFCL and MALT.[44–46] These results suggest that a difference in preferentially affected anatomical site in patients with pcFCL and MALT may exist, at least between Europe and the USA/Asia. By definition, the lower extremities are the most common site in patients with pcDLBCL, leg type. Primary cutaneous small/medium $CD4^+$ T-cell lymphoma is a rare CL entity with an indolent clinical course, which has been shown to preferentially affect the head and neck.[13,47,48] In the present study, the trunk in

Table 5. Incidence pattern of MF/SS by clinical staging

	Study group		
	JSCS	UK[5]	Japan[6]
Total no.	774	1502	100
Surveillance period, year	5	30	25
Clinical stage*			
IA	29.6	29.2	21
IB	39.1	38.8	38
IIA	4.3	2.7	19
IIB	11.1	11.1	8
IIIA	7.4	6.7	6
IIIB	0.9	3.7	0
IVA1	2.2	4.5	2
IVA2	3.6	2.5	3
IVB	1.8	0.9	3

*Clinical stage was identified using the International Society for Cutaneous Lymphomas/European Organization for Research and Treatment of Cancer proposal in 2007. JSCS, Japanese Skin Cancer Society.

addition to the head and neck was the most common site of primary cutaneous small/medium CD4$^+$ T-cell lymphoma. SPTCL is a distinct CL entity, characterized by primarily subcutaneous (mainly fat tissue) infiltration of malignant T lymphocytes with cytotoxic molecules. It predominantly affects the legs.[13,49] Also, we found that the lower extremities were the most commonly affected site in SPTCL (62%). In addition, a female predominance was demonstrated in Japan, as in a previous report.[49]

The present study was conducted to investigate the nationwide incidence patterns of Japanese CL patients, according to the WHO classification. It provides important data about trends in the overall incidence pattern of Japanese CL. In particular, the high prevalences of ATLL and ENKL in Japan are shown, with considerable accuracy. A male predominance was observed in most types of CL, except for SPTCL, ENKL, primary cutaneous CD4$^+$ small/medium T-cell lymphoma and LyP. The present study showed that the proportion of patients in each clinical stage of MF/SS was similar to that in previous studies. In the future, accumulated data from the present registry will allow us to investigate the etiology of varying CL subtypes, and to conduct targeted clinical research based on the characteristics of CL in Japan.

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REFERENCES

1 Groves FD, Linet MS, Travis LB, Devesa SS. Cancer surveillance series: non-Hodgkin's lymphoma incidence by histologic subtype in the United States from 1978 through 1995. *J Natl Cancer Inst* 2000 **92**: 1240–1251.
2 Swerdlow SH, Campo E, Harris NL *et al.*, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. IARC Press, Lyon 2008.
3 Olsen E, Vonderheid E, Pimpinelli N *et al.* Revisions to the staging and classification of mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). *Blood* 2007 **110**: 1713–1722.
4 Kim YH, Willemze R, Pimpinelli N *et al.* TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the Cutaneous Lymphoma Task Force of the European Organization of Research and Treatment of Cancer (EORTC). *Blood* 2007 **110**: 479–484.
5 Agar NS, Wedgeworth E, Crichton S *et al.* Survival outcomes and prognostic factors in mycosis fungoides/Sezary syndrome: validation of the revised International Society for Cutaneous Lymphomas/European Organisation for Research and Treatment of Cancer staging proposal. *J Clin Oncol* 2010 **28**: 4730–4739.
6 Suzuki S, Ito K, Ito M, Kawai K. Prognosis of 100 Japanese patients with mycosis fungoides and Sézary syndrome. *J Dermatol Sci* 2010 **57** (1): 37–43.
7 Bouaziz JD, Bastuji-Garin S, Poszepczynska-Guigné E, Wechsler J, Bagot M. Relative frequency and survival of patients with primary cutaneous lymphomas: data from a single-centre study of 203 patients. *Br J Dermatol* 2006 **154**: 1206–1207.
8 Fujita A, Hamada T, Iwatsuki K. Retrospective analysis of 133 patients with cutaneous lymphomas from a single Japanese medical center between 1995 and 2008. *J Dermatol* 2011 **38**: 524–530.
9 Jenni D, Karpova MB, Seifert B *et al.* Primary cutaneous lymphoma: two-decade comparison in a population of 263 cases from a Swiss tertiary referral centre. *Br J Dermatol* 2011 **164**: 1071–1077.
10 Khamaysi Z, Ben-Arieh Y, Izhak OB, Epelbaum R, Dann EJ, Bergman R. The applicability of the new WHO-EORTC classification of primary cutaneous lymphomas to a single referral center. *Am J Dermatopathol* 2008 **30** (1): 37–44.
11 Liao JB, Chuang SS, Chen HC, Tseng HH, Wang JS, Hsieh PP. Clinicopathologic analysis of cutaneous lymphoma in taiwan: a high frequency of extranodal natural killer/T-cell lymphoma, nasal type, with an extremely poor prognosis. *Arch Pathol Lab Med* 2010 **134**: 996–1002.
12 Park JH, Shin HT, Lee DY *et al.* World Health Organization-European Organization for Research and Treatment of Cancer classification of cutaneous lymphoma in Korea: a retrospective study at a single tertiary institution. *J Am Acad Dermatol* 2012 **67**: 1200–1209.
13 Willemze R, Jaffe ES, Cerroni L *et al.* WHO-EORTC classification for cutaneous lymphomas. *Blood* 2005 **105**: 3768–3785.
14 Assaf C, Gellrich S, Steinhoff M *et al.* Cutaneous lymphomas in Germany: an analysis of the Central Cutaneous Lymphoma Registry of the German Society of Dermatology. *J Dtsch Dermatol Ges* 2007 **5**: 662–668.
15 Bradford PT, Devesa SS, Anderson WF, Toro JR. Cutaneous lymphoma incidence patterns in the United States: a population-based study of 3884 cases. *Blood* 2009 **113**: 5064–5073.
16 Lymphoma Study Group of Japanese Pathologists. The World Health Organization classification of malignant lymphomas in Japan: incidence of recently recognized entities. *Pathol Int* 2000 **50**: 696–702.
17 Ohshima K, Suzumiya J, Kikuchi M. The World Health Organization classification of malignant lymphoma: incidence and clinical prognosis in HTLV-1-endemic area of Fukuoka. *Pathol Int* 2002 **52** (1): 1–12.
18 Olsen EA, Whittaker S, Kim YH *et al.* Clinical end points and response criteria in mycosis fungoides and Sézary syndrome: a consensus statement of the International Society for Cutaneous Lym-

phomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer. *J Clin Oncol* 2011 **29**: 2598–2607.
19 Prince HM, Yap LM, Blum R, McCormack C. Primary cutaneous B-cell lymphomas. *Clin Exp Dermatol* 2003 **28** (1): 8–12.
20 Lee MW Korean Dermatopathology Research Group. Characteristics of cutaneous lymphomas in Korea. *Clin Exp Dermatol* 2003 **28**: 639–646.
21 Uchiyama T, Yodoi J, Sagawa K *et al.* Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* 1977 **50**: 481–492.
22 Poiesz BJ, Ruscetti FW, Gazdar AF *et al.* Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci U S A* 1980 **77** (12): 7415–7419.
23 Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci U S A* 1982 **79**: 2031–2035.
24 Iwanaga M, Chiyoda S, Kusaba E, Kamihira S. Trends in the seroprevalence of HTLV-1 in Japanese blood donors in Nagasaki Prefecture, 2000–2006. *Int J Hematol* 2009 **90**: 186–190.
25 Blattner WA, Kalyanaraman VS, Robert-Guroff M *et al.* The human type-C retrovirus, HTLV, in Blacks from the Caribbean region, and relationship to adult T-cell leukemia/lymphoma. *Int J Cancer* 1982 **30**: 257–264.
26 Nogueira CM, Cavalcanti M, Schechter M, Ferreira OC Jr. Human T lymphotropic virus type I and II infections in healthy blood donors from Rio de Janeiro, Brazil. *Vox Sang* 1996 **70** (1): 47–48.
27 Delaporte E, Dupont A, Peeters M *et al.* Epidemiology of HTLV-I in Gabon (Western Equatorial Africa). *Int J Cancer* 1988 **42**: 687–689.
28 Shimoyama M. Diagnosis criteria and classification of clinical subtypes of adult T-cell leukemialymphoma: a report from the Lymphoma Study Group. *Br J Haematol* 1991 **79**: 428–437.
29 Bunn PA Jr, Schechter GP, Jaffe E *et al.* Clinical course of retrovirus-associated adult T-cell lymphoma in the United States. *N Engl J Med* 1983 **309**: 257–264.
30 Setoyama M, Katahira Y, Kanzaki T. Clinicopathologic analysis of 124 cases of adult T-cell leukemia/lymphoma with cutaneous manifestations: the smouldering type with skin manifestations has a poorer prognosis than previously thought. *J Dermatol* 1999 **26**: 785–790.
31 Chan HL, Su IJ, Kuo T *et al.* Cutaneous manifestations of adult T-cell leukemia/lymphoma. Report of three different forms. *J Am Acad Dermatol* 1985 **13** (2 Pt 1): 213–219.
32 Sawada Y, Hino R, Hama K *et al.* Type of skin eruption is an independent prognostic indicator for adult T-cell leukemia/lymphoma. *Blood* 2011 **117**: 3961–3967.
33 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**: 206–214.
34 Quintanilla-Martinez L, Franklin JL, Guerrero I *et al.* Histological and immunophenotypic profile of nasal NK/T cell lymphomas from Peru: high prevalence of p53 overexpression. *Hum Pathol* 1999 **30**: 849–855.
35 Yoon SO, Suh C, Lee DH *et al.* Distribution of lymphoid neoplasms in the Republic of Korea: analysis of 5318 cases according to the World Health Organization classification. *Am J Hematol* 2010 **85**: 760–764.
36 Yasukawa K, Kato N, Kodama K, Hamasaka A, Hata H. The spectrum of cutaneous lymphomas in Japan: a study of 62 cases based on the World Health Organization Classification. *J Cutan Pathol* 2006 **33**: 487–491.
37 Nagasawa T, Miwa H, Nakatsuka S, Itami S, Yoshikawa K, Aozasa K. Characteristics of cutaneous lymphomas in Osaka, Japan (1988-1999) based on the European Organization for Research and Treatment of Cancer classification. *Am J Dermatopathol* 2000 **22**: 510–514.
38 Bunn PA Jr, Huberman MS, Whang-Peng J *et al.* Prospective staging evaluation of patients with cutaneous T-cell lymphomas. Demonstration of a high frequency of extracutaneous dissemination. *Ann Intern Med* 1980 **93**: 223–230.
39 Sausville EA, Eddy JL, Makuch RW *et al.* Histopathologic staging at initial diagnosis of mycosis fungoides and the Sézary syndrome. Definition of three distinctive prognostic groups. *Ann Intern Med* 1988 **109**: 372–382.
40 Zackheim HS, Amin S, Kashani-Sabet M *et al.* Prognosis in cutaneous T-cell lymphoma by skin stage: long-term survival in 489 patients. *J Am Acad Dermatol* 1999 **40**: 418–425.
41 van Doorn R, Van Haselen CWvan Voorst Vader PC *et al.* Mycosis fungoides: disease evolution and prognosis of 309 Dutch patients. *Arch Dermatol* 2000 **136**: 504–510.
42 Kim YH, Liu HL, Mraz-Gernhard S *et al.* Long-term outcome of 525 patients with mycosis fungoides and Sézary syndrome: clinical prognostic factors and risk for disease progression. *Arch Dermatol* 2003 **139**: 857–866.
43 Li C, Inagaki H, Kuo TT, Hu S, Okabe M, Eimoto T. Primary cutaneous marginal zone B-cell lymphoma: a molecular and clinicopathologic study of 24 asian cases. *Am J Surg Pathol* 2003 **27**: 1061–1069.
44 Hoefnagel JJ, Vermeer MH, Jansen PM *et al.* Primary cutaneous marginal zone B-cell lymphoma: clinical and therapeutic features in 50 cases. *Arch Dermatol* 2005 **141**: 1139–1145.
45 Senff NJ, Hoefnagel JJ, Jansen PM *et al.* Reclassification of 300 primary cutaneous B-Cell lymphomas according to the new WHO-EORTC classification for cutaneous lymphomas: comparison with previous classifications and identification of prognostic markers. *J Clin Oncol* 2007 **25**: 1581–1587.
46 Zinzani PL, Quaglino P, Pimpinelli N *et al.* Prognostic factors in primary cutaneous B-cell lymphoma: the Italian Study Group for Cutaneous Lymphomas. *J Clin Oncol* 2006 **24**: 1376–1382.
47 Garcia-Herrera A, Colomo L, Camós M *et al.* Primary cutaneous small/medium CD4+ T-cell lymphomas: a heterogeneous group of tumors with different clinicopathologic features and outcome. *J Clin Oncol* 2008 **26**: 3364–3371.
48 Beltraminelli H, Leinweber B, Kerl H, Cerroni L. Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoma: a cutaneous nodular proliferation of pleomorphic T lymphocytes of undetermined significance? A study of 136 cases. *Am J Dermatopathol* 2009 **31**: 317–322.
49 Willemze R, Jansen PM, Cerroni L *et al.* Subcutaneous panniculitis-like T-cell lymphoma: definition, classification, and prognostic factors: an EORTC Cutaneous Lymphoma Group Study of 83 cases. *Blood* 2008 **111**: 838–845.

Vaccination With NY-ESO-1 Overlapping Peptides Mixed With Picibanil OK-432 and Montanide ISA-51 in Patients With Cancers Expressing the NY-ESO-1 Antigen

Hisashi Wada, Midori Isobe,† Kazuhiro Kakimi,‡ Yu Mizote,† Shingo Eikawa,† Eiichi Sato,§ Nagio Takigawa,‖ Katsuyuki Kiura,‖ Kazuhide Tsuji,¶ Keiji Iwatsuki,¶ Makoto Yamasaki,* Hiroshi Miyata,* Hirokazu Matsushita,‡ Heiichiro Udono,# Yasuyuki Seto,** Kazuhiro Yamada,†† Hiroyoshi Nishikawa,‡‡ Linda Pan,§§ Ralph Venhaus,§§ Mikio Oka,† Yuichiro Doki,* and Eiichi Nakayama‖‖*

Summary: We conducted a clinical trial of an NY-ESO-1 cancer vaccine using 4 synthetic overlapping long peptides (OLP; peptides #1, 79–108; #2, 100–129; #3, 121–150; and #4, 142–173) that include a highly immunogenic region of the NY-ESO-1 molecule. Nine patients were immunized with 0.25 mg each of three 30-mer and a 32-mer long NY-ESO-1 OLP mixed with 0.2 KE Picibanil OK-432 and 1.25 mL Montanide ISA-51. The primary endpoints of this study were safety and NY-ESO-1 immune responses. Five to 18 injections of the NY-ESO-1 OLP vaccine were well tolerated. Vaccine-related adverse events observed were fever and injection site reaction (grade 1 and 2). Two patients showed stable disease after vaccination. An NY-ESO-1-specific humoral immune response was observed in all patients and an antibody against peptide #3 (121–150) was detected firstly and strongly after vaccination. NY-ESO-1 CD4 and CD8 T-cell responses were elicited in these patients and their epitopes were identified. Using a multifunctional cytokine assay, the number of single or double cytokine-producing cells was increased in NY-ESO-1-specific CD4 and CD8 T cells after vaccination. Multiple cytokine-producing cells were observed in PD-1 (−) and PD-1 (+) CD4 T cells. In conclusion, our study indicated that the NY-ESO-1 OLP vaccine mixed with Picibanil OK-432 and Montanide ISA-51 was well tolerated and elicited NY-ESO-1-specific humoral and CD4 and CD8 T-cell responses in immunized patients.

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From the *Department of Gastroenterological Surgery, Graduate School of Medicine; ‡‡Experimental Immunology, Immunology Frontier Research Center, Osaka University, Suita, Osaka; †Department of Respiratory Medicine, Kawasaki Medical School; ‖‖Faculty of Health and Welfare, Kawasaki University of Medical Welfare, Kurashiki; Departments of ‖Medicine; ¶Dermatology; #Immunology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Okayama; Departments of ‡Immunotherapeutics; **Gastrointestinal Surgery, University of Tokyo Hospital, Bunkyo-ku; §Department of Pathology, Tokyo Medical University, Shinjuku-ku; ††Department of Gastroenterological Surgery, The Cancer Institute Hospital, Koto-ku, Tokyo, Japan; and §§Ludwig Institute for Cancer Research, New York, NY.

The study was registered in the University hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) Clinical Trial (Unique trial number: UMIN000001857) on April 7, 2009 (UMIN-CTRURL: http://www.umin.ac.jp/ctr/index.htm).

Reprints: Hisashi Wada, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan. E-mail: hwada@gesurg.med.osaka-u.ac.jp.

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The NY-ESO-1 antigen was originally identified in esophageal cancer by serological expression cloning (SEREX) using autologous patient serum.[1] NY-ESO-1 expression is observed in a wide range of human malignancies, but the expression is restricted to germ cells in the testis in normal adult tissues.[2] Therefore, NY-ESO-1 has emerged as a prototype of a class of cancer/testis (CT) antigens.

Numerous cancer vaccine strategies are under development.[3,4] For patients with hormone-resistant prostate cancer, a dendritic cell (DC) vaccine has recently been approved.[5] The therapy is based on loading autologous DCs ex vivo with a lysate of a cultured prostate cancer cell line transfected with the genes of acid phosphatase and GM-CSF, with subsequent administration to patients to induce specific T-cell responses. However, its clinical efficacy seems to be limited.[6,7] Cancer vaccines using recombinant proteins and peptides are thought to involve DCs in vivo and have advantages in that materials are easy to secure, there is little toxicity, and there are no complex regulatory matters when compared with cell therapy.[8] As cancer vaccines using short peptides showed only limited efficacy, cancer vaccines using synthetic long peptides have been introduced.[4,9–11] Synthetic peptides of 25–50 amino acids are internalized and processed by DCs efficiently, and presented the antigens on MHC class I and II for T cells.[12,13] Maturation of DCs is associated with upregulation of costimulatory molecules on their surfaces and is crucial for efficient induction of T-cell responses. Adjuvants such as TLR ligands induce DC maturation and strongly augment the immunogenicity of cancer vaccines.[3,14]

Detection of pathogen-associated molecular patterns by the pattern recognition receptors on DCs and activation of subsequent signaling induce specific CD4 and CD8 T-cell responses.[15,16] Thus, the stimulatory effect of innate immunity on adaptive immune responses is useful for cancer vaccines. TLRs, nucleotide-binding oligomerization domain–like receptors, the retinoic acid-inducible gene-I-like or RIG-like receptors, and the C-type lectin receptors are the 4 known families of pattern recognition receptors.[17] In this study, we immunized patients with advanced cancers expressing the NY-ESO-1 antigen with 30–32-mer NY-ESO-1 overlapping long peptides (OLP), and Picibanil

OK-432 and Montanide ISA-51 as immunomodulators. OK-432 is a penicillin-killed and lyophilized preparation of a low-virulence strain (Su) of *Streptococcus pyogenes*.[9,18,19] It was developed as a nonspecific immune stimulant, TLR2, 4, and/or 9 agonist. OK-432 induces various cytokines including tumor necrosis factor-α (TNF-α), interferon (IFN), interleukin (IL)-2, and IL-6.[20] Recently, it was shown that in vivo administration of OK-432 overcame regulatory T-cell suppression in mice.[21] The vaccine also induced efficient NY-ESO-1 immunity in patients.

MATERIALS AND METHODS

NY-ESO-1 OLP Vaccine

NY-ESO-1 OLPs [peptide #1: NY-ESO-1 79–108 (GARGPESRLLEFYLAMPFATPMEAELARRS), peptide #2: NY-ESO-1 100–129 (MEAELARRSLAQDAPPLP VPGVLLKEFTVS), peptide #3: NY-ESO-1 121–150 (VLLKEFTVSGNILTIRLTAADHRQLQLSIS), and peptide #4: NY-ESO-1 142–173 (HRQLQLSISSCLQQL SLLMWITQCFLPVFLAQ)] were synthesized by Multiple Peptide Systems (San Diego, CA). The vaccine, consisting of 1 mg of NY-ESO-1 OLP including 0.25 mg each of the 4 peptides, 0.2 KE OK-432 (Picibanil; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) and 1.25 mL ISA-51(Montanide; Seppic, Paris, France), was emulsified under sterile conditions. Synthesis, production, formulation, and packaging of the investigational agent were in accordance with current Good Manufacturing Practices and met the applicable criteria for use in humans.

Study Design

A pilot, open-label, multi-institutional clinical trial of the NY-ESO-1 OLP vaccine was designed to evaluate the safety, immune response, and clinical response. Patients eligible for entry were those who had advanced cancers that were refractory to standard therapy and expressed NY-ESO-1 as assessed by immunohistochemistry (IHC), a performance status of 0, 1, or 2, were 20 years old or above, had a life expectancy of 4 months or more, and did not have impaired organ function. Patients ineligible were those who were positive for HIV antibodies, had multiple cancers, autoimmune disease, serious allergy history, or active brain metastasis, or received chemotherapy, systemic steroid, or immunosuppressive therapy in the last 4 weeks. Nine patients, including 7 patients with esophageal cancer, a patient with lung cancer, and a patient with malignant melanoma, were enrolled in a washout period after surgery, chemotherapy or radiation therapy. The vaccine was administered subcutaneously once every 2 (esophageal cancer patients) or 3 (lung cancer and malignant melanoma patients) weeks to achieve better performance status in esophageal cancer patients to complete a cycle of 6 vaccinations. Four weeks after the last administration, the safety, immune response, and clinical response were evaluated. Thereafter, the vaccine was administered additionally. The 9 patients received 5–18 immunizations. Clinical response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST ver1.1)[22] and the immune-related response criteria (irRC).[23] Safety was evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events ver.3.0 (NCI-CTCAE ver.3.0) (http://ctep.cancer.gov/). The protocol was approved by the Ethics Committee of Osaka, Tokyo and Okayama Universities in light of the Declaration of Helsinki. Written informed consent was obtained from each patient before enrollment in the study. The study was conducted in compliance with Good Clinical Practice. The study was registered in the University hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) Clinical Trial (unique trial number: UMIN000001857) on April 7, 2009 (UMIN-CTRURL: http://www.umin.ac.jp/ctr/index.htm).

Blood Samples

Peripheral blood was drawn from the patients at baseline, at each time point of immunization, and 4 weeks after the last immunization. Peripheral blood mononuclear cells (PBMCs) and plasma were isolated by density gradient centrifugation using lymphoprep (AXIS-SHIELD Poc AS, Oslo, Norway). A CD8 T-cell–enriched population was obtained from PBMCs using CD8 microbeads with a large-scale column and a magnetic device (Miltenyi Biotec, Auburn, CA). A CD4 T-cell–enriched population was then obtained from the residual cells using CD4 microbeads. The final residual cells were used as a CD4-depleted and CD8-depleted population. The 3 populations were stored in liquid N_2 until use. HLA typing of PBMCs was performed by sequence-specific oligonucleotide probing and sequence-specific priming of genomic DNA using standard procedures.

NY-ESO-1 18-Mer Series Peptides

The following series of 28 overlapping NY-ESO-1 18-mer peptides spanning the protein were synthesized: 1–18, 7–24, 13–30, 19–36, 25–42, 31–48, 37–54, 43–60, 49–66, 55–72, 61–78, 67–84, 73–90, 79–96, 85–102, 91–108, 97–114, 103–120, 109–126, 115–132, 121–138, 127–144, 133–150, 139–156, 145–162, 149–166, 153–170, and 156–173. A 30-mer peptide, 151–180, was also synthesized. These 29 peptides (NY-ESO-1 18-mer series peptides) were synthesized using standard solid-phase methods based on N-(9-fluorenyl)-methoxycarbonyl (Fmoc) chemistry on a Multiple Peptide Synthesizer (AMS422; ABIMED, Langenfeld, Germany) at Okayama University.

ELISA

Recombinant NY-ESO-1 protein was prepared as described previously.[24] Recombinant protein (1 μg/mL) or peptide (10 μg/mL) in a coating buffer (15 mM Na_2CO_3, 30 mM $NaHCO_3$, pH 9.6) was adsorbed onto 96-well Polysorp immunoplates (Nunc, Roskilde, Denmark) and incubated overnight at 4°C. Plates were washed with PBS and blocked with 200 μL/well of 5% FCS/PBS for 1 hour at room temperature. After washing, 100 μL of serially diluted plasma was added to each well and incubated for 2 hours at room temperature. After extensive washing, horseradish peroxidase-conjugated goat anti-human pan-IgG, IgG_1, IgG_2, IgG_3, IgG_4, or IgM (Medical & Biological Laboratories, Nagoya, Japan) was added to the wells, and the plates were incubated for 1 hour at room temperature. After washing and development, absorbance at 490 nm was read. Recombinant murine Akt protein[24] and ovalbumin (albumin from chicken egg white; Sigma, St. Louis, MO) were used as control proteins.

In Vitro Stimulation of CD4 and CD8 T Cells

Frozen cells were thawed and resuspended in AIM-V (Invitrogen, Carlsbad, CA) medium supplemented with 5% heat-inactivated pooled human serum (CM), and kept at room temperature for 2 hours. CD4-enriched and CD8-enriched populations (2×10^6) were cultured with irradiated

(30 Gy), autologous CD4-depleted and CD8-depleted PBMCs (2×10^6) in the presence of 29 NY-ESO-1 18-mer series peptides in 2 mL of CM supplemented with 10 U/mL rIL-2 (Takeda Chemical Industries, Osaka, Japan) and 10 ng/mL rIL-7 (Peprotech, London, UK) in a 24-well culture plate at 37°C in a 5% CO_2 atmosphere for 12 days. For the second stimulation, 1×10^6 instead of 2×10^6 responder cells were used in the culture described above. For nonspecific immune activation, T cells were stimulated with 50 ng/mL PMA and 1 μg/mL ionomycin with GolgiStop (BD Biosciences, Franklin Lakes, NJ) for 6 hours at 37°C.

IFN-γ Catch Assay

Responder CD4 or CD8 T cells (5×10^4) from the stimulation culture were cultured for 4 hours with autologous EBV-B cells (5×10^4) pulsed with mixed, or one of the 29 NY-ESO-1 18-mer series peptides. The cells were then treated with a bispecific CD45 and IFN-γ antibody (IFN-γ catch reagent; 2 μL) for 5 minutes on ice. The cells were diluted in AIM-V medium (3 mL) and placed on a slow rotating device (Miltenyi Biotec, Bergisch Gladbach, Germany) to allow IFN-γ secretion at 37°C in a 5% CO_2 atmosphere. After incubation for 1 hour, the cells were washed with cold buffer and treated with PE-conjugated anti-IFN-γ (detection reagent), and FITC-conjugated anti-CD4 or anti-CD8 mAb. After incubation for 10 minutes at 4°C, the cells were washed and analyzed by FACS Canto II (BD Biosciences).

IHC

IHC was performed as described previously.[9] E978[25] and EMR8-5 (Funakoshi, Tokyo, Japan) mAbs were used to analyze NY-ESO-1 and HLA class I expression, respectively. The reaction was evaluated as + + + (> 50% stained cells), + + (50%–25% stained cells), + (25%–5% stained cells), and − (< 5% stained cells).

Intracellular Staining (ICS) of Cytokines

CD4 or CD8 T cells were washed and treated with Cytofix and Perm Wash (BD Biosciences) according to the manufacturer instructions to stain them with IFN-γ-APC (BD Biosciences), IL-2-APC (BD Biosciences), IL-5-PE (BD Biosciences), IL-10-APC (BioLegend, San Diego, CA), IL-17 A-PerCP-Cy5.5 (eBioscience, San Diego, CA), and/or TNF-α-FITC (eBioscience) intracellularly. For Foxp3 staining, a human Foxp3 staining kit, Alexa 488 (BD Biosciences), was used. Cells were analyzed using a FACS Canto II (BD Biosciences) and FlowJo software (Tree Star, Ashland, OR).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis

To amplify the *NY-ESO-1* cDNA segment, primers specific for *NY-ESO-1* were designed as described previously.[26] Primers for RT-PCR were: *NY-ESO-1 5′-AGT TCTACCTCGCCATGCCT-3′* (forward), *5′-TCCTCCT CCAGCGACAAACAA-3′* (reverse), *GAPDH 5′-ACCACA GTCCATGCCATCAC-3′* (forward), *5′-TCCACCACC CTGTTGCTGTA-3′* (reverse). The amplification program for *NY-ESO-1* was 1 minute at 94°C, 1 minute at 60°C, and 1.5 minutes at 72°C for 35 cycles after denaturing at 94°C for 1 minute. These cycles were followed by a 10-minute elongation step at 72°C. The amplification program for *GAPDH* was 1 minute at 94°C, 1 minute at 66°C, and 1.5 minutes at 72°C for 30 cycles after denaturing at 94°C for 1 minute. These cycles were followed by a 10-minute elongation step at 72°C. The PCR products were analyzed on a 1.3% agarose gel.

RESULTS

Patient Characteristics

Nine patients with advanced cancers expressing the NY-ESO-1 antigen were enrolled (Table 1). Seven were esophageal cancer patients. One was a malignant melanoma patient and 1 was a lung cancer patient. Eight patients completed a cycle of 6 vaccinations. One esophageal cancer patient, P-5, did not complete a cycle of vaccination due to disease progression and was given only 5 vaccinations, but was included in the analysis. Two patients, P-6 and P-7, were given additional vaccinations because of stable disease (SD) during treatment (Table 2).

Safety

Injection site reactions were observed in all patients (Table 1). The reaction was grade 1 in 7 patients and grade 2 with induration in 2 patients, but resolved without any treatment several months after vaccination. Fever (grade 1) was observed in 3 patients. No other adverse events either related or nonrelated to the vaccine were observed. The vaccine was well tolerated.

Monitoring of Humoral Immune Response

Serum antibodies against full-length NY-ESO-1 protein and also against 4 individual NY-ESO-1 OLP (peptides #1, 79–108; #2, 100–129; #3, 121–150; and #4, 142–173) used for the vaccine were investigated (Fig. 1). Three patients, P-2, P-3, and P-4, had antibodies against the NY-ESO-1 protein at the baseline (seropositive). Of those, P-2 and P-3 showed no antibodies against the 4 peptides, whereas P-4 showed antibodies against peptide #1 (79–108),

TABLE 1. Patient Characteristics

Patients	Age/Sex	Cancer	Vaccination	Vaccine-related Toxicity
P-1	69/M	Malignant melanoma	6	Injection site reaction (grade 1)
P-2	55/M	Lung cancer (adenocarcinoma)	6	Fever (grade 1), injection site reaction (grade 1)
P-3	66/M	Esophageal cancer (squamous cell carcinoma)	6	Injection site reaction (grade 1)
P-4	70/M	Esophageal cancer (squamous cell carcinoma)	6	Injection site reaction (grade 1)
P-5	58/M	Esophageal cancer (squamous cell carcinoma)	5	Injection site reaction (grade 1)
P-6	67/M	Esophageal cancer (squamous cell carcinoma)	18	Injection site reaction (grade 1)
P-7	74/M	Esophageal cancer (squamous cell carcinoma)	8	Injection site reaction (grade 1)
P-8	69/M	Esophageal cancer (squamous cell carcinoma)	6	Fever (grade 1), injection site reaction (grade 2)
P-9	70/F	Esophageal cancer (squamous cell carcinoma)	6	Fever (grade 1), injection site reaction (grade 2)

TABLE 2. Immune Responses and Tumor Responses After Vaccination With the NY-ESO-1 OLP Peptide

Patients	IHC*		Antibody‡		CD4§		CD8§		Target Tumor		Clinical Response‖ (Duration)¶
	MHC	NY-ESO-1	Pre	Post	Pre	Post	Pre	Post	Region	Total Diameter (mm)	
P-1	ND	+ †	−	+ + +	−	+	−	+	abLN + axLN	48	PD
P-2	+ +	+	−	+ + +	−	+ +	−	+ +	meLN	24	PD
P-3	+ +	+	−	+ + +	−	+ + +	−	+ + +	abLN	32	PD
P-4	+ + +	+ + +	+ +	+ + +	ND	ND	ND	ND	liver	20	PD
P-5	+ +	+ + +	−	+ + +	−	−	−	−	pleura	28	PD
P-6	+ +	+ + +	−	+ + +	−	+	−	−	abLN + liver	33	SD (7 mo)
P-7	+ +	+	−	+ + +	−	+ +	−	+	neLN	18	SD (4 mo)
P-8	+	+ + +	−	+ + +	−	+	−	+	pleura	30	PD
P-9	+	+ +	−	+ +	ND	ND	ND	ND	pleura	25	PD

*IHC was performed using EMR8-5 mAb for MHC class I (MHC) and E957 mAb for NY-ESO-1. IHC-positive cells: + + + > 50%; 50% ≥ + + > 25%; 25% ≥ + > 5%; 5% ≥ −.
†NY-ESO-1 expression of P-1 was determined by RT-PCR.
‡Antibody response was determined by ELISA (see Materials and methods section) using OD values at 25× dilution for each OLP peptide and at 100× or 1600× dilution for NY-ESO-1 protein. Antibody response shown here represents OD for NY-ESO-1 OLP: + + + > 2; 2 ≥ + + > 0.5; 0.5 ≥ + > 0.1; 0.1 ≥ −.
§CD4 and CD8 T-cell responses were determined by an IFN-γ catch assay with the cells stimulated in vitro once (1°IVS). IFN-γ-positive cells: + + + > 10%; 10% ≥ + + > 5%; 5% ≥ + > 1%; 1% ≥ −. The response was confirmed by cells stimulated in vitro twice (2°IVS).
‖Clinical response was evaluated by RECIST and irRC.
¶SD duration was measured from the start of the immunization until the patients were defined as PD by RECIST (P-6) or removed due to disease progression (P-7).
Ab indicates abdomen; ax, axilla; LN, lymph node; me, mediastinum; ne, neck; ND, not done; PD, progressive disease; SD, stable disease.

but not against the others. After vaccination, antibodies against peptide #3 (121–150) were detected firstly and strongly in all patients. Antibodies against peptide #4 (142–173) were also detected in 7 of 9 patients. In contrast, the antibody responses against peptides #1 (79–108) and #2 (100–129) were relatively weak. The antibody responses against the NY-ESO-1 protein also increased in parallel, or with a delay, compared with antibody responses against the peptides in all patients. Notably, in patients P-6 and P-7 who showed SD, strong antibody responses against the peptides and the protein were observed. The dominant Ig subtypes were IgG_1 and IgG_3. An increased IgM response against peptide #3 after vaccination was observed in 6 patients.

Monitoring of CD4 and CD8 T-Cell Responses

CD4 and CD8 T cells purified from PBMCs using antibody-coated magnetic beads were cultured for 12 days with a mixture of 29 NY-ESO-1 18-mer series peptides spanning the entire NY-ESO-1 protein [stimulated in vitro once (1°IVS)] and assayed for IFN-γ production by an IFN-γ catch assay after stimulation with a mixture of 29 NY-ESO-1 18-mer series peptides for 4 hours. CD4 and CD8 T cells harvested from 1°IVS were again cultured in a similar way to 1°IVS, except using 1×10^5 instead of 1×10^6 responder cells [stimulated in vitro twice (2°IVS)] and assayed by an IFN-γ catch assay to confirm weak response. As shown in Figure 2, an increase in the CD4 T-cell response was observed in all 7 patients investigated in 1°IVS. In contrast, an increase in the CD8 T-cell response was observed in 5 of 7 patients in 1°IVS. A CD8 T-cell response was detected after 2°IVS in 2 patients (P-5 and P-6), who showed only a marginal response in 1°IVS. The Supplementary Figure (Supplemental Digital Content 1, http://links.lww.com/JIT/A313) shows the representative results of the IFN-γ catch assay for P-3 and P-7.

Next, the peptides recognized by CD4 and CD8 T cells were investigated by an IFN-γ catch assay using cells stimulated in 2°IVS and testing against individual 29 NY-ESO-1 18-mer series peptides. As shown in Figure 3, CD4 T cells dominantly recognized 18-mer peptides #15 and #21 and their adjacent peptides. A CD8 T-cell response was observed against various peptides, including 18-mer peptides #15 and #21, which were recognized relatively dominantly. In the case of CD8, a response against peptides (1–78) not included in the vaccine preparation was also observed. The Supplementary Table (Supplemental Digital Content 2, http://links.lww.com/JIT/A314) shows patient HLA.

$Foxp3^+$ CD4 cells were also examined by ICS. As shown in Figure 2, a decrease after vaccination was observed in 4 of 6 patients investigated. In contrast, a slight increase followed by a decrease was observed in 2 patients.

Clinical Observation

As shown in Table 2, patients P-6 and P-7 showed SD during vaccinations and were given additional 12 and 2 vaccinations, respectively. The other 7 patients showed PD during vaccinations. There was no discrepancy in evaluation between RECIST and irRC and no evidence of clinical benefit after immunizations. The results of immunomonitoring and clinical responses are summarized in Table 2. As previously described, in patients P-6 and P-7 who showed SD, strong antibody responses against the peptides and the protein were observed.

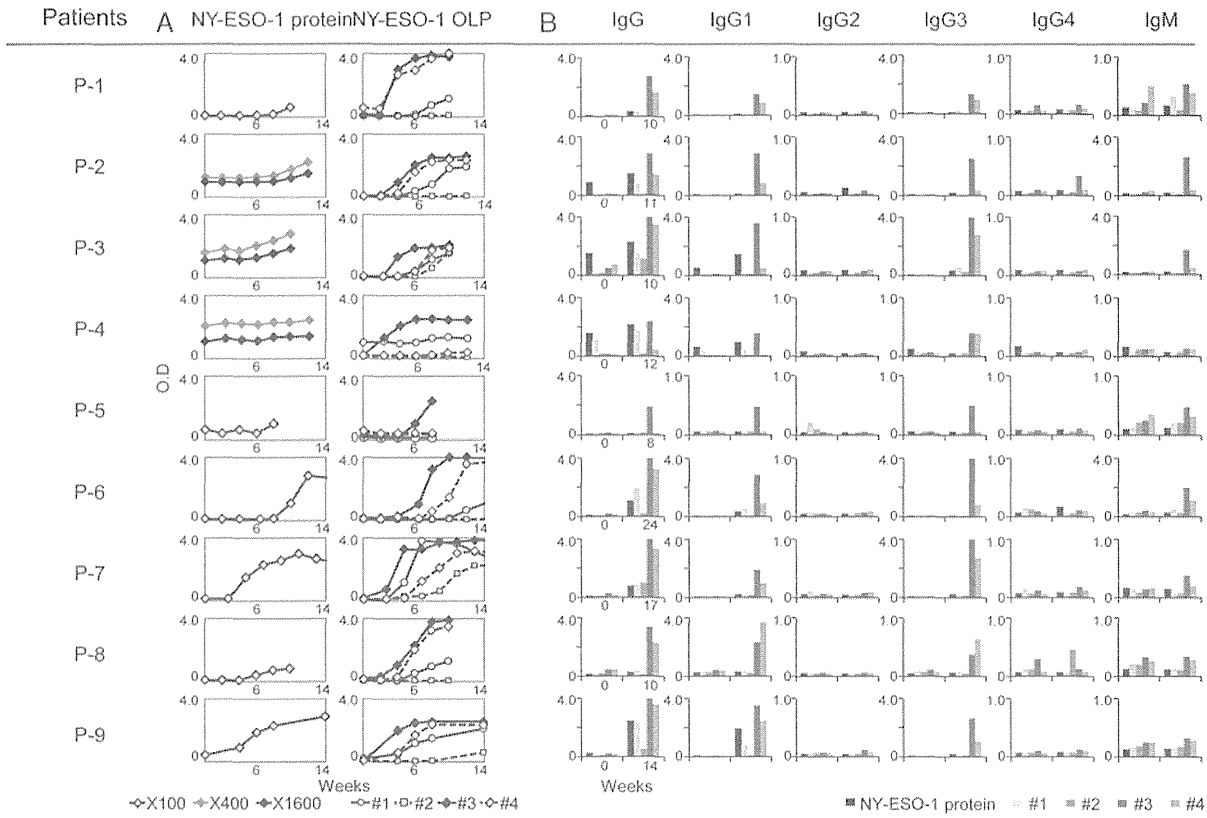

FIGURE 1. Immunomonitoring of humoral immune responses after NY-ESO-1 OLP vaccination. NY-ESO-1 antibody responses against recombinant NY-ESO-1 protein for each of the NY-ESO-1 OLP peptides were evaluated using plasma obtained before and after vaccination with NY-ESO-1 OLP from the 9 patients. A, Whole IgG antibody responses against recombinant NY-ESO-1 protein or each peptide included in the NY-ESO-1 OLP vaccine at a dilution of 1:100 (open diamond) or 1:1600 (closed diamond) for the protein and at a dilution of 1:25 for the peptides at the baseline (week 0) and at each vaccination. B, Whole IgG, IgG_1, IgG_2, IgG_3, IgG_4, and IgM antibody responses against the protein or each peptide at a dilution of 1:25 at the baseline and at the indicated week after vaccination.

Multiple Cytokine Production by CD4 and CD8 T Cells After Stimulation With NY-ESO-1 OLP

CD4 and CD8 T cells from patient P-3 and P-7 PBMCs purified by antibody-coated magnetic beads were stimulated in vitro with a mixture of 29 NY-ESO-1 18-mer series peptides for 12 days as previously shown and assayed for IL-2, TNF-α, and IFN-γ-producing cells by ICS using FACS. As shown in Figure 4, an increase in the number of single cytokine-producing cells was observed in CD4 and CD8 T cells after vaccination. A slight increase in double cytokine-producing cells was also observed. No triple cytokine-producing cells were observed. In addition, an increase in CD4 T cells producing IL-5 or IL-17, but not IL-10, was observed after vaccination, suggesting activation of Th2 and Th17, as well as Th1 (Fig. 4B).

Nonspecific Immune Activation by the Vaccine

In addition, nonspecific immune activation by the vaccine preparation was investigated using CD4 T cells from patient P-7 obtained before and after vaccination, and stimulated with PMA/ionomycin by ICS. As shown in Figures 4C and D, while no triple cytokine-producing cells were observed in either PD-1 (–) or PD-1 (+) CD4 T cells at day 0, these cells appeared even after a single vaccination (day 15). No further increase in these cells was observed in CD4 T cells obtained after the 10th vaccination (day 78). No significant change in PD-1 (–) or PD-1 (+) CD4 T-cell populations was observed during the vaccination period.

DISCUSSION

In this study, we showed that an NY-ESO-1 OLP vaccine with Picibanil OK-432 and Montanide ISA-51 was safe and induced NY-ESO-1 humoral and cellular immune responses in all patients. In our previous study on cancer vaccines with NY-ESO-1 protein[24–29] and the NY-ESO-1f peptide,[9] NY-ESO-1-specific humoral immune responses appeared to be useful as an immunological marker to predict the clinical responses of the patients vaccinated. This study showed that of the 4 peptides used, the antibody response against peptide #3 (121–150) was the most useful for monitoring than the responses against other peptides. The dominant epitope recognized by CD4 T cells (18-mer peptide #21, NY-ESO-1 121–138) in Figure 3 is included in the sequence of peptide #3. It is interesting to note that, the epitope peptide recognized by the antibody induced spontaneously in patients with NY-ESO-1-expressing tumors or in patients vaccinated with the NY-ESO-1 protein was NY-ESO-1 91–108, and was not included in peptide #3.[23] A mixture of four 30–32-mer long peptides used for the vaccine included a hydrophobic sequence located in a region approximately 121–170 amino acid, which is normally buried in the molecule[23] and therefore altered the immunological dominance of the antibody response to NY-ESO-1.

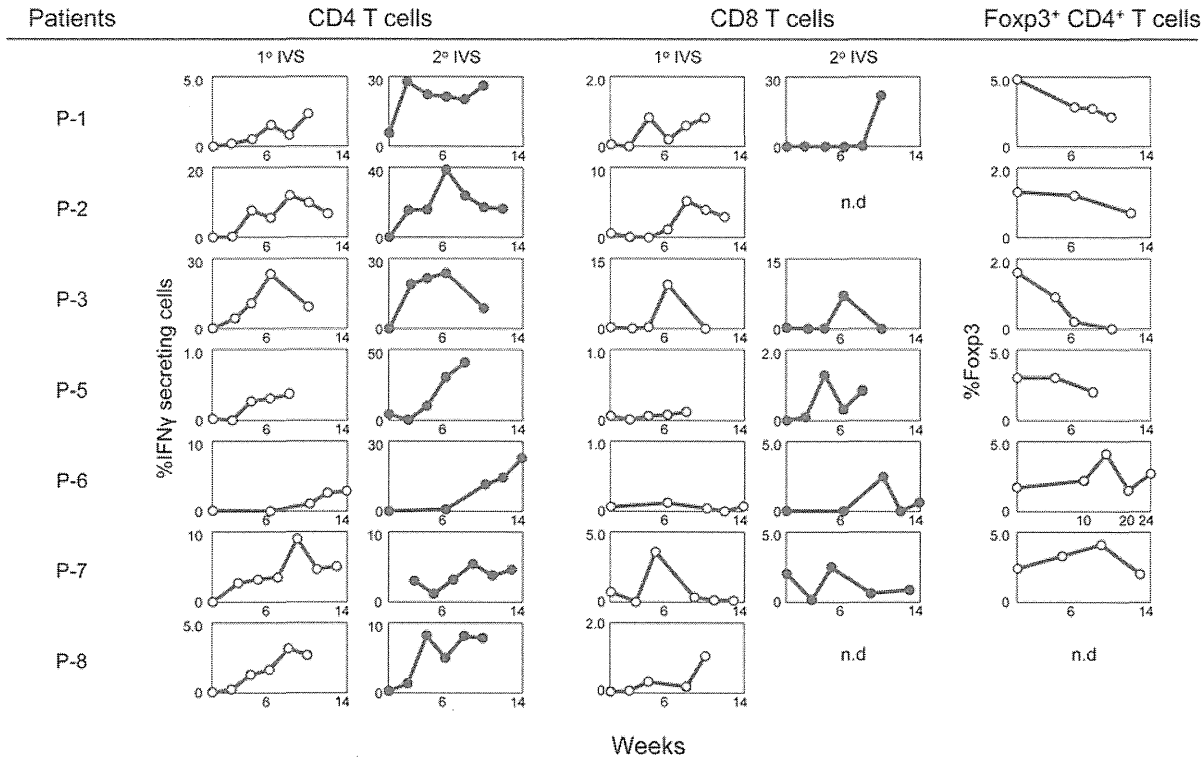

FIGURE 2. Immunomonitoring of CD4 and CD8 T-cell responses and Treg cells. CD4 and CD8 T cells were stimulated with 29 NY-ESO-1 18-mer series peptides once (1°IVS) or twice (2° IVS) and the net percentage of interferon (IFN)-γ-secreting cells was evaluated by an IFN-γ catch assay using bispecific CD45 and an IFN-γ antibody. Foxp3$^+$ CD4 Tregs were evaluated by ICS. The data are plotted with time. The analysis was conducted by FACS Canto II. ND indicates not done.

We used an IFN-γ catch assay to detect antigen-specific cellular responses using PBMCs in immunomonitoring. The assay can potentially give rise to false-positive reactions due to neighboring cells picking up IFN-γ by the bystander effect. However, it could be avoided by carefully performing the assay using a larger volume in a limited

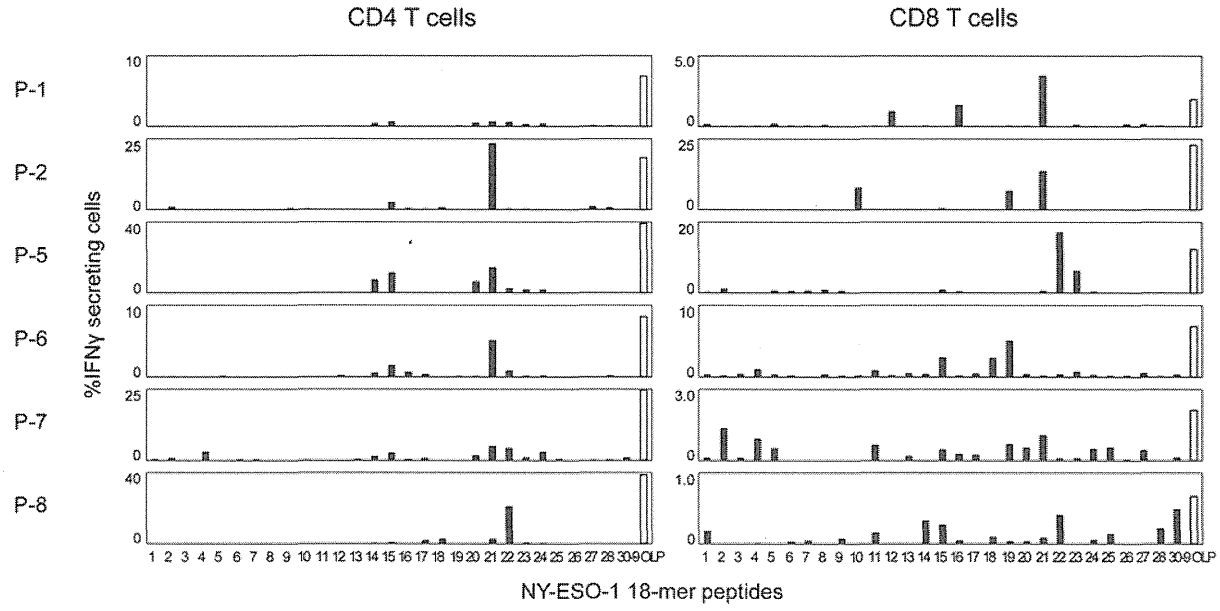

FIGURE 3. Epitope peptides recognized by CD4 and CD8 T cells. Using CD4 and CD8 T cells stimulated twice for 26 days with irradiated (30 Gy) autologous CD4-depleted and CD8-depleted PBMCs in the presence of 29 NY-ESO-1 18-mer series peptides, the epitope peptides recognized were determined by an interferon (IFN)-γ catch assay. In the assay, the cells were stimulated with autologous EBV-B cells pulsed with each 29 NY-ESO-1 18-mer peptide individually. The analysis was conducted by FACS Canto II.

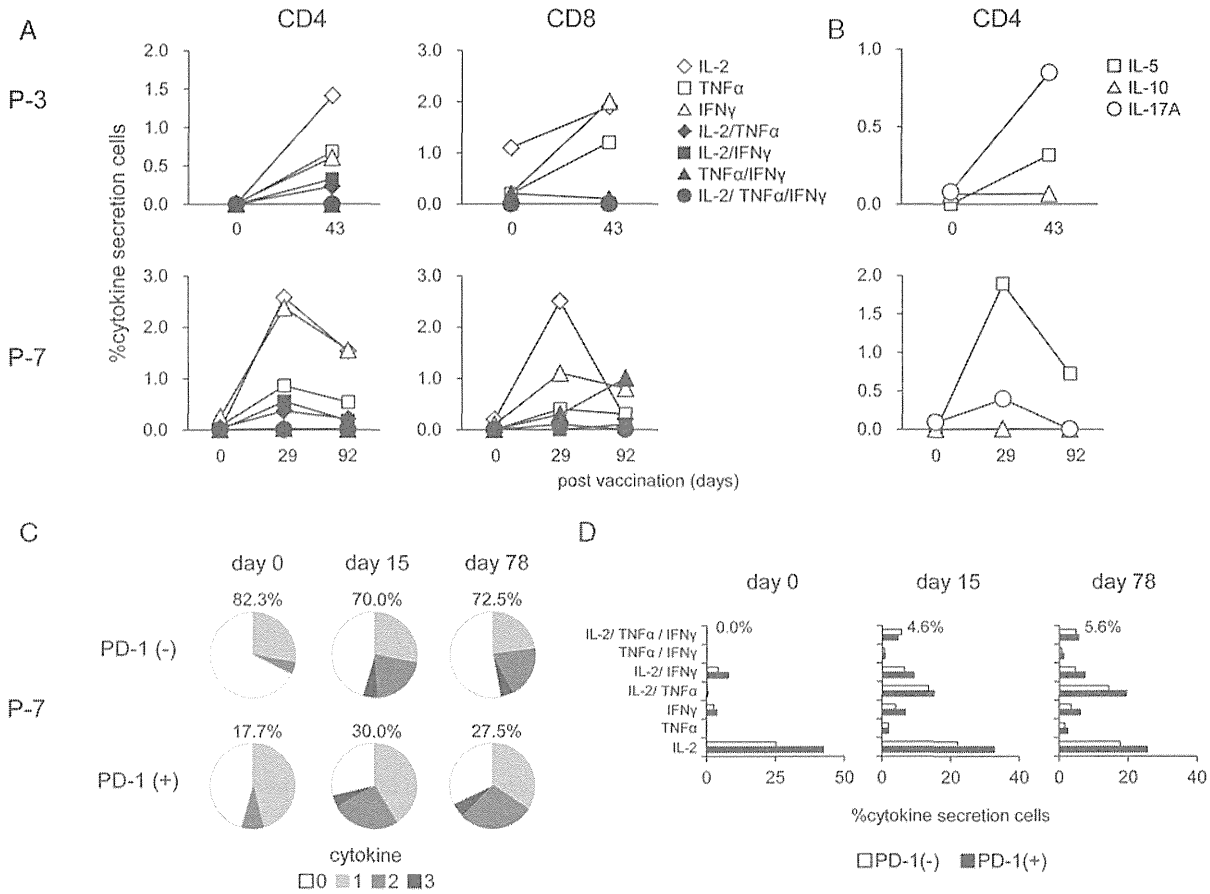

FIGURE 4. Multiple cytokine production by CD4 and CD8 T cells and nonspecific immune activation after NY-ESO-1 OLP vaccination. Multiple cytokine production by CD4 and CD8 T cells stimulated with a mixture of 29 NY-ESO-1 18-mer series peptides (A, B) or PMA/ionomycin (C, D) was analyzed. The cells from patients P-3 (A, B) and P-7 (A, B and C, D) obtained before and after vaccination were assayed for IL-2, TNF-α, and interferon (IFN)-γ-producing cells (A, C, D) or IL-5, IL-10, and IL-17A-producing cells (B) by intracellular staining. Frequency of single, double, and triple cytokine-producing cells in CD4 and CD8 T cells (A, B) or in PD-1 (–) or (+) CD4 T cells (C, D) before and after vaccination are depicted.

concentration of IFN-γ. The assay was highly reproducible and sensitive compared with intracytoplasmic staining or an ELISPOT assay. In the ELISPOT assay, it should be noted that the response resulting from a minor CD4 T-cell population contaminated in a purified CD8 population can sometimes make the interpretation of results difficult, especially after stimulation with longer peptides. Induction of CD4 T-cell responses was observed in all patients vaccinated and their increase during the vaccination period was consistent with the results in our previous study on a cancer vaccine with NY-ESO-1 protein and NY-ESO-1f peptide. For induction of NY-ESO-1-specific CD4 T cells, overlapping peptides appeared to be much more efficient than protein.[10] In contrast, induction of CD8 T-cell responses was similarly observed in all patients vaccinated with NY-ESO-1 OLP. However, the responses were relatively weak and fluctuated a lot during vaccination compared with the CD4 T-cell response. In our previous study with NY-ESO-1 protein, even a patient with a tumor that almost completely disappeared showed only a marginal CD8 T-cell response in PBMCs.[25,29] We observed some Foxp3$^+$ CD4 T cells infiltrating tumor tissue from this patient. In this study, we detected Foxp3$^+$ CD4 T cells in PBMCs from all patients analyzed during vaccination with NY-ESO-1 OLP. No increase in regulatory T cells was observed during vaccination in PBMCs. It is possible that those cells suppress CD8 T-cell responses in tumor microenvironments. Future studies combining cancer vaccines and inhibition of regulatory T-cell function will be intriguing.

TLRs are expressed either on the cell surface (TLR1, 2, 4, 5, 6, and 10) or on the membrane of intracellular organelles such as endosomes (TLR3, 7, 8, and 9). The bacterial CpG motif is the ligand for TLR9. Viral single-stranded RNAs are ligands for TLR7 and 8, and double-stranded viral RNAs such as PolyI:C are ligands of TLR3. Molecular patterns of extracellular microbes are recognized by the cell surface-expressed TLR1, 2, 4, 5, 6, and 10.[30] OK-432 is a ligand for TLR2, 4, and/or 9.[9] Triggering of TLR signaling leads to the activation of nuclear factor κB, activating protein-1, and/or IRF3, which results in secretion of type 1 IFNs and/or proinflammatory cytokines such as IL-1β, IL-12, and TNF-α.[17] We used OK-432 as an immunemodulator for the NY-ESO-1f peptide, CHP-NY-ESO-1 and CHP-HER2 cancer vaccine and observed efficient induction of tumor antigen-specific immune responses.[9,18,31] Sabbatini et al[32] used an NY-ESO-1 OLP vaccine with or without poly-ICLC in ovarian cancer patients. They observed that an efficient antibody response against NY-ESO-1 OLP was