PFS and OS differences between the two arms. Thus, although a tendency towards prolongation of PFS in the combination arm was observed in the present study, this was not confirmed. Second, the treatment after the study protocol, including allo-HCT and mogamulizumab, varied among the patients. Because the use of mogamulizumab for relapsed/refractory ATL was approved in Japan during the study period, the patients, including those in the chemotherapy alone arm, may have a chance to receive this drug. Both of these factors may affect the OS (Chihara *et al*, 2013).

In conclusion, although mLSG15 plus mogamulizumab was found to be associated with a potentially less favourable safety profile, particularly for infectious and skin-related events, the majority of the AEs were manageable. The %CR was higher with combination therapy. Accordingly, this combination treatment appears to be a better option for managing patients with newly diagnosed aggressive ATL. Further clinical studies are necessary to evaluate the survival parameters in patients treated with chemotherapy plus mogamulizumab and to determine a more suitable combination regimen.

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Author contributions

T.I., K.U., K.Y., N.U., A.U., K.T., S.A. and R.U. contributed to the conception and design of the study; T.I., T.J., S.T., H.S., K.U., K.Y., N.U., Y.S., K.N., A.U., K.T., H.F., K. Ishitsuka, S.Y., N.T., Y.M., K. Imada and T.M. contributed to the acquisition of data; T.I., K.T., S.A., M.T. and R.U. analysed and interpreted the data; all authors drafted and reviewed the manuscript and approved the final version for submission.

Conflicts of interest

T.I. has received honoraria and travel grants from Kyowa Hakko Kirin, and research funding from Kyowa Hakko Kirin, Chugai, Bayer and Celgene, and has served on the speakers bureau for Kyowa Hakko Kirin. T.J. has received honoraria and travel grants from Kyowa Hakko Kirin. S.T. has received travel grants and research funding from Kyowa Hakko Kirin. H.S. has served on the speakers bureau for Kyowa Hakko Kirin. K.Y. has a consultancy/advisory role with Kyowa Hakko Kirin and Novartis, has received honoraria from Kyowa Hakko Kirin, Novartis, Takeda and Janssen, and has received research funding from Kyowa Hakko Kirin, Novartis, Pfizer and ARIAD. Y.S. has received honoraria and research funding from Kyowa Hakko Kirin. K.N. has received honoraria from Kyowa Hakko Kirin. A.U. has received honoraria and research funding from Kyowa Hakko Kirin. K.T. has received honoraria from Kyowa Hakko Kirin and research funding from Kyowa Hakko Kirin, Celgene, Eisai, Solasia Pharma and Mundipharma. S.Y. has received honoraria and research funding from Kyowa Hakko Kirin. Y.M. has received honoraria, travel grants and research funding from Kyowa Hakko Kirin. K. Imada has received research funding from Kyowa Hakko Kirin. S.A. is employed by Kyowa Hakko Kirin, and is a stock owner. M.T. has a consultancy/advisory role with Kyowa Hakko Kirin, and has received honoraria from Kyowa Hakko Kirin. R.U. has a consultancy/advisory role with Mundipharma, and has received honoraria, travel grants and research funding from Kyowa Hakko Kirin and Chugai, and has served on the speakers bureau for Kyowa Hakko Kirin. The remaining authors declare no competing financial interests.

Appendix I

List of the review committees and medical experts who participated in this trial:

1 Junji Suzumiya, Shimane University Hospital
2 Takashi Terauchi, Research Centre for Cancer Prevention and Screening National Cancer Centre
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4 Junichi Tsukada, University of Occupational and Environmental Health
5 Koichi Nakata, University of Occupational and Environmental Health
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7 Hiroshi Inagaki, Nagoya City University Graduate School of Medical Sciences
8 Koichi Ohshima, Kurume University School of Medicine
9 Michinori Ogura, Nagoya Daini Red Cross Hospital
10 Tetsuo Nagatani, Hachioji Medical Centre of Tokyo Medical University
11 Akimichi Morita, Nagoya City University Graduate School of Medical Sciences
12 Kazunari Yamaguchi, Institute of Molecular Embryology and Genetics, Kumamoto University
13 Yasuaki Yamada, Nagasaki University Graduate School of Biomedical Sciences
14 Shuichi Hanada, National Hospital Organization Kagoshima Medical Centre.

References

Chihara, D., Ito, H., Matsuda, T., Katanoda, K., Shibata, A., Taniguchi, S., Utsunomiya, A., Sobue, T. & Matsuo, K. (2013) Association between decreasing trend in the mortality of adult T-cell leukemia/lymphoma and allogeneic hematopoietic stem cell transplants in Japan: analysis of Japanese vital statistics and Japan Society for Hematopoietic Cell Transplantation (JSHCT). *Blood Cancer Journal*, **3**, e159.

Hishizawa, M., Kanda, J., Utsunomiya, A., Taniguchi, S., Eto, T., Moriuchi, Y., Tanosaki, R., Kawano, F., Miyazaki, Y., Masuda, M., Nagafuji, K., Hara, M., Takanashi, M., Kai, S., Atsuta, Y., Suzuki, R., Kawase, T., Matsuo, K., Nagamura-Inoue, T., Kato, S., Sakamaki, H., Morishima, Y., Okamura, J., Ichinohe, T. & Uchiyama, T. (2010) Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study. *Blood*, **116**, 1369–1376.

Ishida, T. & Ueda, R. (2011) Immunopathogenesis of lymphoma: focus on CCR4. *Cancer Science*, **102**, 44–50.

Ishida, T., Utsunomiya, A., Iida, S., Inagaki, H., Takatsuka, Y., Kusumoto, S., Takeuchi, G., Shimizu, S., Ito, M., Komatsu, H., Wakita, A., Eimoto, T., Matsushima, K. & Ueda, R. (2003) Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. *Clinical Cancer Research*, **9**, 3625–3634.

Ishida, T., Inagaki, H., Utsunomiya, A., Takatsuka, Y., Komatsu, H., Iida, S., Takeuchi, G., Eimoto, T., Nakamura, S. & Ueda, R. (2004) CXC chemokine receptor 3 and CC chemokine receptor 4 expression in T-cell and NK-cell lymphomas with special reference to clinicopathological significance for peripheral T-cell lymphoma, unspecified. *Clinical Cancer Research*, **10**, 5494–5500.

Ishida, T., Hishizawa, M., Kato, K., Tanosaki, R., Fukuda, T., Taniguchi, S., Eto, T., Takatsuka, Y., Miyazaki, Y., Moriuchi, Y., Hidaka, M., Akashi, K., Uike, N., Sakamaki, H., Morishima, Y., Kato, K., Suzuki, R., Nishiyama, T. & Utsunomiya, A. (2012a) Allogeneic hematopoietic stem cell transplantation for adult T-cell leukemia-lymphoma with special emphasis on preconditioning regimen: a nationwide retrospective study. *Blood*, **120**, 1734–1741.

Ishida, T., Joh, T., Uike, N., Yamamoto, K., Utsunomiya, A., Yoshida, S., Saburi, Y., Miyamoto, T., Takemoto, S., Suzushima, H., Tsukasaki, K., Nosaka, K., Fujiwara, H., Ishitsuka, K., Inagaki, H., Ogura, M., Akinaga, S., Tomonaga, M., Tobinai, K. & Ueda, R. (2012b) Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase II study. *Journal of Clinical Oncology*, **30**, 837–842.

Ishida, T., Ito, A., Sato, F., Kusumoto, S., Iida, S., Inagaki, H., Morita, A., Akinaga, S. & Ueda, R. (2013) Stevens-Johnson Syndrome associated with mogamulizumab treatment of adult T-cell leukemia/lymphoma. *Cancer Science*, **104**, 647–650.

Ishii, T., Ishida, T., Utsunomiya, A., Inagaki, A., Yano, H., Komatsu, H., Iida, S., Imada, K., Uchiyama, T., Akinaga, S., Shitara, K. & Ueda, R. (2010) Defucosylated humanized anti-CCR4 monoclonal antibody KW-0761 as a novel immunotherapeutic agent for adult T-cell leukemia/lymphoma. *Clinical Cancer Research*, **16**, 1520–1531.

Iwanaga, M., Watanabe, T. & Yamaguchi, K. (2012) Adult T-cell leukemia: a review of epidemiological evidence. *Frontiers in Microbiology*, **3**, 322.

Jacobs, J.F., Nierkens, S., Figdor, C.G., de Vries, I.J. & Adema, G.J. (2012) Regulatory T cells in melanoma: the final hurdle towards effective immunotherapy? *Lancet Oncology*, **13**, e32–e42.

Matsuoka, M. & Jeang, K.T. (2007) Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. *Nat Reviews Cancer*, **7**, 270–280.

Michels-van Amelsfort, J.M., Walter, G.J. & Taams, L.S. (2011) $CD4^+$ $CD25^+$ regulatory T cells in systemic sclerosis and other rheumatic diseases. *Expert Review of Clinical Immunology*, **7**, 499–514.

Miyara, M., Yoshioka, Y., Kitoh, A., Shima, T., Wing, K., Niwa, A., Parizot, C., Taflin, C., Heike, T., Valeyre, D., Mathian, A., Nakahata, T., Yamaguchi, T., Nomura, T., Ono, M., Amoura, Z., Gorochov, G. & Sakaguchi, S. (2009) Functional delineation and differentiation dynamics of human $CD4^+$ T cells expressing the FoxP3 transcription factor. *Immunity*, **30**, 899–911.

Nakagawa, M., Nakagawa-Oshiro, A., Karnan, S., Tagawa, H., Utsunomiya, A., Nakamura, S., Takeuchi, I., Ohshima, K. & Seto, M. (2009) Array comparative genomic hybridization analysis of PTCL-U reveals a distinct subgroup with genetic alterations similar to lymphoma-type adult T-cell leukemia/lymphoma. *Clinical Cancer Research*, **15**, 30–38.

Ogata, M., Satou, T., Kawano, R., Yoshikawa, T., Ikewaki, J., Kohno, K., Ando, T., Miyazaki, Y., Ohtsuka, E., Saburi, Y., Kikuchi, H., Saikawa, T. & Kadota, J. (2011) High incidence of cytomegalovirus, human herpesvirus-6, and Epstein-Barr virus reactivation in patients receiving cytotoxic chemotherapy for adult T cell leukemia. *Journal of Medical Virology*, **83**, 702–709.

Ogura, M., Ishida, T., Hatake, K., Taniwaki, M., Ando, K., Tobinai, K., Fujimoto, K., Yamamoto, K., Miyamoto, T., Uike, N., Tanimoto, M., Tsukasaki, K., Ishizawa, K., Suzumiya, J., Inagaki, H., Tamura, K., Akinaga, S., Tomonaga, M. & Ueda, R. (2014) Multicenter phase II study of mogamulizumab (KW-0761), a defucosylated anti-cc chemokine receptor 4 antibody, in patients with relapsed peripheral T-cell lymphoma and cutaneous T-cell lymphoma. *Journal of Clinical Oncology*, **32**, 1157–1163.

Pocock, S.J. & Simon, R. (1975) Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics*, **31**, 103–115.

Shimoyama, M. (1991) Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma. A report from the Lymphoma Study Group (1984–87). *British Journal of Haematology*, **79**, 428–437.

Shinkawa, T., Nakamura, K., Yamane, N., Shoji-Hosaka, E., Kanda, Y., Sakurada, M., Uchida, K., Anazawa, H., Satoh, M., Yamasaki, M., Hanai, N. & Shitara, K. (2003) The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. *Journal of Biological Chemistry*, **278**, 3466–3473.

Simon, R., Wittes, R.E. & Ellenberg, S.S. (1985) Randomized phase II clinical trials. *Cancer Treatment Reports*, **69**, 1375–1381.

Sugiyama, D., Nishikawa, H., Maeda, Y., Nishioka, M., Tanemura, A., Katayama, I., Ezoe, S., Kanakura, Y., Sato, E., Fukumori, Y., Karbach, J., Jäger, E. & Sakaguchi, S. (2013) Anti-CCR4 mAb selectively depletes effector-type FoxP3 $CD4^+$ regulatory T cells, evoking antitumor immune responses in humans. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 17945–17950.

Tsukasaki, K., Utsunomiya, A., Fukuda, H., Shibata, T., Fukushima, T., Takatsuka, Y., Ikeda, S., Masuda, M., Nagoshi, H., Ueda, R., Tamura, K., Sano, M., Momita, S., Yamaguchi, K., Kawano, F., Hanada, S., Tobinai, K., Shimoyama, M., Hotta, T. & Tomonaga, M. (2007) VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. *Journal of Clinical Oncology*, **25**, 5458–5464.

Tsukasaki, K., Hermine, O., Bazarbachi, A., Ratner, L., Ramos, J.C., Harrington, W. Jr, O'Mahony, D., Janik, J.E., Bittencourt, A.L., Taylor, G.P., Yamaguchi, K., Utsunomiya, A., Tobinai, K. & Watanabe, T. (2009) Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: a proposal from an international consensus meeting. *Journal of Clinical Oncology*, **27**, 453–459.

Uchiyama, T., Yodoi, J., Sagawa, K., Takatsuki, K. & Uchino, H. (1977) Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood*, **50**, 481–492.

Waldmann, T.A., Greene, W.C., Sarin, P.S., Saxinger, C., Blayney, D.W., Blattner, W.A., Goldman, C.K., Bongiovanni, K., Sharrow, S. & Depper, J.M. (1984) Functional and phenotypic comparison of human T cell leukemia/lymphoma virus positive adult T cell leukemia with human T cell leukemia/lymphoma virus negative Sézary leukemia, and their distinction using anti-Tac. Monoclonal antibody identifying the human receptor for T cell growth factor. *The Journal of Clinical Investigation*, **73**, 1711–1718.

Yamada, Y., Tomonaga, M., Fukuda, H., Hanada, S., Utsunomiya, A., Tara, M., Sano, M., Ikeda,

S., Takatsuki, K., Kozuru, M., Araki, K., Kawano, F., Niimi, M., Tobinai, K., Hotta, T. & Shimoyama, M. (2001) A new G-CSF-supported combination chemotherapy, LSG15, for adult T-cell leukaemia-lymphoma: Japan Clinical Oncology Group Study 9303. *British Journal of Haematology*, **113**, 375–382.

Yamamoto, K., Utsunomiya, A., Tobinai, K., Tsukasaki, K., Uike, N., Uozumi, K., Yamaguchi, K., Yamada, Y., Hanada, S., Tamura, K., Nakamura, S., Inagaki, H., Ohshima, K., Kiyoi, H., Ishida, T., Matsushima, K., Akinaga, S., Ogura, M., Tomonaga, M. & Ueda, R. (2010) Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. *Journal of Clinical Oncology*, **28**, 1591–1598.

Yano, H., Ishida, T., Inagaki, A., Ishii, T., Kusumoto, S., Komatsu, H., Iida, S., Utsunomiya, A. & Ueda, R. (2007) Regulatory T-cell function of adult T-cell leukemia/lymphoma cells. *International Journal of Cancer*, **120**, 2052–2057.

Yoshie, O., Fujisawa, R., Nakayama, T., Harasawa, H., Tago, H., Izawa, D., Hieshima, K., Tatsumi, Y., Matsushima, K., Hasegawa, H., Kanamaru, A., Kamihira, S. & Yamada, Y. (2002) Frequent expression of CCR4 in adult T-cell leukemia and human T-cell leukemia virus type 1-transformed T cells. *Blood*, **99**, 1505–1511.

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Case Report

Fatal reactivation of hepatitis B virus infection in a patient with adult T-cell leukemia–lymphoma receiving the anti-CC chemokine receptor 4 antibody mogamulizumab

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We report an adult T-cell leukemia–lymphoma (ATL) patient suffering from fatal reactivation of hepatitis B virus (HBV) infection after treatment with the anti-CC chemokine receptor 4 (CCR4) monoclonal antibody, mogamulizumab. HBV reactivation occurred without liver damage in this hepatitis B surface antigen (HBsAg) negative patient, who was seropositive for antibodies against the viral core and surface antigens at baseline, after two cycles of CHOP regimen (cyclophosphamide, doxorubicin, vincristine and prednisolone) followed by six cycles of THP-COP regimen (cyclophosphamide, pirarubicin, vincristine and prednisolone). Unexpectedly, mogamulizumab monotherapy for relapsed CCR4 positive ATL induced sudden and fatal liver failure due to HBV reactivation, despite antiviral prophylaxis with entecavir. This clinical course may not only offer important suggestions to prevent critical HBV reactivation in HBsAg positive cancer patients who receive immune-enhancing drugs such as anti-CCR4 antibody, but also provide a clue to understanding the pathogenesis of HBV reactivation following systemic chemotherapy.

Key words: antiviral prophylaxis, CC chemokine receptor type 4, hepatitis B virus, mogamulizumab, reactivation

INTRODUCTION

REACTIVATION OF HEPATITIS B virus (HBV) infection is a potentially critical complication in cancer patients following systemic chemotherapy.[1–4] It can occur not only in patients seropositive for hepatitis B surface antigen (HBsAg),[5,6] but also in those with resolved HBV infection who are seronegative for HBsAg but seropositive for antibodies against hepatitis B core antigen (anti-HBc) and/or antibodies against HBsAg (anti-HBs).[3,4,7–9] Antiviral prophylaxis is recommended by some guidelines to prevent HBV reactivation-related hepatitis and death for HBsAg-positive patients.[10–13]

Adult T-cell leukemia–lymphoma (ATL) is an aggressive peripheral T-cell neoplasm and human T-cell leukemia virus type 1 (HTLV-1) plays a role in its pathogenesis. Recently, the anti-CC chemokine receptor 4 (CCR4) monoclonal antibody, mogamulizumab, was developed and introduced into the management of ATL in Japan.[14–18]

We report here fatal HBV reactivation in an ATL patient after mogamulizumab monotherapy, despite antiviral prophylaxis.

CASE REPORT

A 72-YEAR-OLD JAPANESE man was admitted with the major complaint of bilateral cervical lymphadenopathy. The laboratory findings showed no abnormal lymphocytes in the peripheral blood, but elevated levels of

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Conflict of interest: S. K. received research funding from Kyowa Hakko Kirin, and honoraria from Kyowa Hakko Kirin and Bristol-Myers Squibb. Y. T. received research funding from Bristol-Myers Squibb, and honoraria from Kyowa Hakko Kirin and Bristol-Myers Squibb. T. I. received research funding from Kyowa Hakko Kirin, Bayer Pharma and Celgene, and honoraria from Kyowa Hakko Kirin. M. M. received research funding from Gilead Science. R. U. received research funding from Kyowa Hakko Kirin. S. I. received research funding from Kyowa Hakko Kirin.

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lactate dehydrogenase (492 U/L) and soluble interleukin-2 receptor (89 903 U/L). He was seropositive for anti-HTLV-1 antibody. A computed tomographic scan revealed systemic lymphadenopathy, hepatomegaly, a low-density lesion in the spleen, and the presence of pleural effusion and ascites. A diagnosis of lymphoma-type ATL was made on the basis of histopathological examination of a cervical lymph node biopsy and the monoclonal integration of HTLV-1, detected by Southern blotting.

He received two cycles of CHOP regimen (cyclophosphamide, doxorubicin, vincristine and prednisolone), followed by five cycles of THP-COP regimen (cyclophosphamide, pirarubicin, vincristine and prednisolone), and achieved best objective response of partial response to ATL. However, his right cervical lymph node was enlarged just before the sixth cycle of THP-COP regimen and progressive disease of CCR4 positive ATL was judged by the histopathological examination of a second lymph node biopsy (Fig. 1).

He was seronegative for HBsAg before initiating CHOP chemotherapy but neither anti-HBc nor anti-HBs was measured at baseline. When he received a transfusion of red blood cells after the third cycle of THP-COP regimen, his serological HBV markers, which we examined using a preserved sample, were as follows: HBsAg negative (0.02 IU/mL, chemiluminescent immunoassay [CLIA]), anti-HBc positive (9.32 s/co, CLIA) and anti-HBs-positive (68.8 mIU/mL, CLIA). However, the reappearance of HBsAg and seropositivity of hepatitis B e-antigen were confirmed after the sixth cycle of THP-COP regimen, when a high HBV DNA level was detected (>9.1 log copies/mL) by real-time polymerase chain reaction assay before initiating salvage chemotherapy for ATL using mogamulizumab (Fig. 1). Liver damage did not develop, although a very high level of HBV DNA was detected at that point.

He began to receive weekly mogamulizumab monotherapy for relapsed CCR4 positive ATL and received anti-HBV treatment with entecavir (0.5 mg/day) at the same time, according to Japanese guidelines for preventing HBV reactivation-related hepatitis. His cervical lymphadenopathy improved, however, sudden and severe liver damage (alanine aminotransferase, 2410 U/L; total bilirubin, 6.01 mg/dL) developed after the third cycle of mogamulizumab (Figs 1,2). A diagnosis of acute liver failure was made on sustained jaundice with hepatic coma and the decline to less than 40% of prothrombin time, according to Japanese criteria,[19] despite the daily administration of entecavir and Stronger Neo-Minophagen C (40 mg/day). He was transferred to another hospital for intensive care, including plasma exchange, and his HBV DNA levels decreased to 3.6 log copies/mL immediately (Fig. 1), which indicated that entecavir was

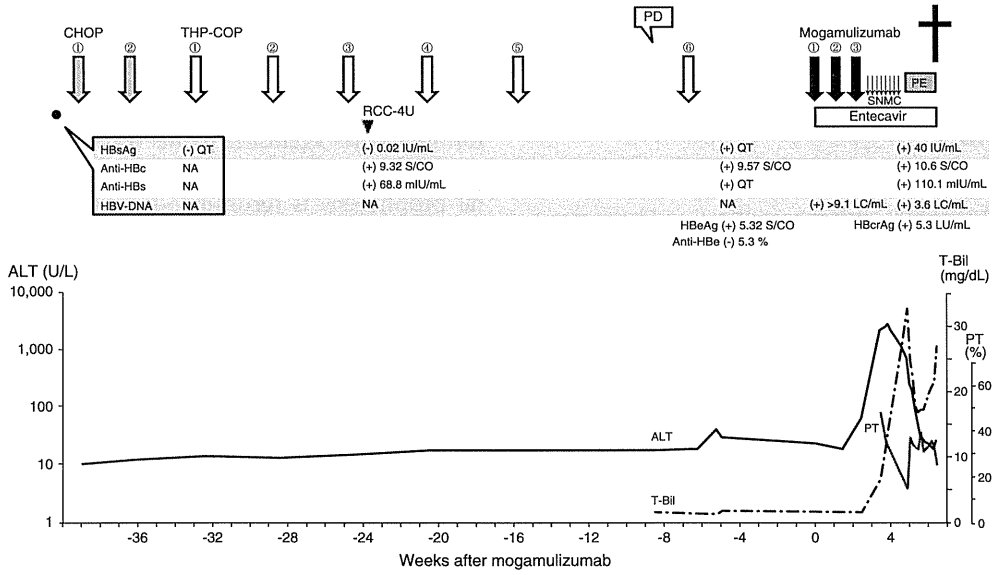

Figure 1 Clinical course of a patient with adult T-cell leukemia–lymphoma before and after treatment with the anti-CC chemokine receptor 4 monoclonal antibody mogamulizumab. ALT, alanine aminotransferase; anti-HBc, antibody against hepatitis core antigen; anti-HBe, antibody against hepatitis B e-antigen; anti-HBs, antibody against HBsAg; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisolone; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LC/mL, log copies/mL; LU/mL, log U/mL; NA, not available; PD, progressive disease; PE, plasma exchange; PT, prothrombin time; QT, qualitative test; RCC, red cells concentrates; sIL-2R, soluble interleukin-2 receptor; SNMC, Stronger Neo-Minophagen C; T-Bil, total bilirubin; THP-COP, pirarubicin, vincristine and prednisolone.

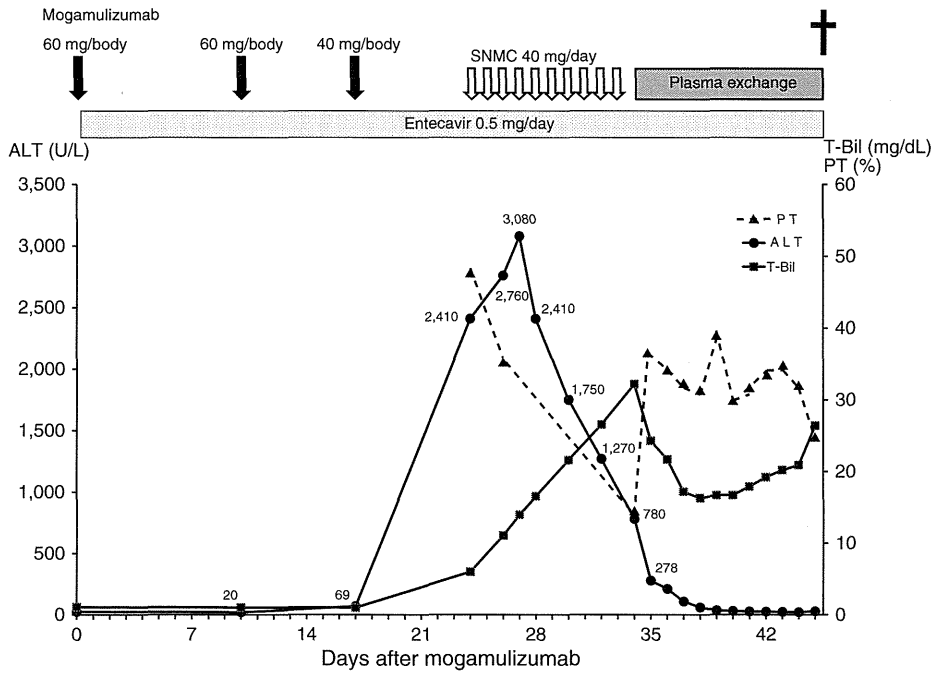

Figure 2 Detailed clinical course of fatal HBV reactivation after mogamulizumab. ALT, alanine aminotransferase; HBV, hepatitis B virus; SNMC, Stronger Neo-Minophagen C; PT, prothrombin time; T-Bil, total bilirubin.

effective in suppressing the replication of HBV DNA. There was no evidence of the emergence of resistance to this antiviral drug at that time. However, acute liver failure progressed rapidly and he died approximately 7 weeks after the first administration of mogamulizumab.

Retrospective analysis using a sample stored 2 weeks after hepatitis onset (at 5 weeks after the first administration of mogamulizumab) showed that he was seropositive for HBsAg (40 IU/mL, CLIA), seropositive for anti-HBc (10.6 s/co, CLIA), seropositive for anti-HBs (110.1 mIU/mL, CLIA) and seropositive for HBc-related antigen (5.3 log U/mL, CLIA). Furthermore, analysis showed that the virus was HBV genotype C, without any mutations in the precore region or basal core promoter, but a HBsAg escape mutation (G145K) was found. This retrospective analysis was approved by the institutional review board of Amagasaki Central Hospital and Nagoya City University.

DISCUSSION

THIS CASE IS the first report of HBV-related death in an ATL patient who received mogamulizumab, despite antiviral prophylaxis with entecavir before hepatitis onset, according to some guidelines.[10–13] Mogamulizumab is a humanized anti-CCR4 monoclonal antibody, which targets CCR4 molecules expressed not only on tumor cells of ATL, but also normal T-helper type 2 and regulatory T cells.[14,15,20] Mogamulizumab has been demonstrated to be effective for CCR4 positive relapsed/refractory ATL or peripheral T-cell lymphoma,[16,18,21] however, Stevens–Johnson syndrome, a seriously adverse skin event, has been reported to occur, partly because of an enhanced cellular immune response following a remarkable reduction in the number of regulatory T cells.[22]

In this case, the initial systemic chemotherapy (CHOP or THP-COP regimen), possibly in addition to the highly immunocompromised situation caused by ATL itself,[20,23] might have led to the reappearance of HBsAg and an abrupt increase of serum HBV DNA, and HBV reactivation might have occurred before the administration of mogamulizumab. Although HBV reactivation has been reported to be a rare complication following anti-CD20 monoclonal antibody, rituximab-free chemotherapy,[8,9] this clinical course suggested that regular HBV DNA monitoring-guided pre-emptive antiviral therapy is necessary to prevent HBV reactivation in such patients.[3,10] Interestingly, liver damage associated with HBV reactivation did not manifest, even when a very high HBV DNA level was detected. Acute liver failure with hepatic coma developed suddenly after the administration of mogamulizumab and the patient died of hepatitis B in spite of intensive care.

As to why the sudden acute liver failure occurred after mogamulizumab, this anti-CCR4 antibody should rapidly deplete not only CCR4 positive ATL cells, a certain subset of which functions as regulatory T cells, but also CCR4 positive endogenous non-ATL regulatory T cells.[16,18,23] We surmise that this depletion resulted in a rapid provocation of the patient's immune system, especially the cellular immune response to HBV-infected hepatocytes, leading to acute liver failure and HBV-related death. HBV reactivation has been reported to occur most frequently in cancer patients after completion of systemic chemotherapy and recovery of the immune response.[3] Antiviral prophylaxis is recommended by some guidelines to prevent HBV reactivation-related hepatitis and death in HBsAg positive patients before initiating systemic chemotherapy.[10–13] Recently, a randomized controlled trial demonstrated that the prophylactic use of entecavir significantly decreased the risk of HBV reactivation in HBsAg positive patients with low viral loads (serum HBV DNA levels <3 log copies/mL), who received rituximab-containing chemotherapy, compared with the prophylactic use of lamivudine.[24] However, there is limited evidence of prevention of HBV reactivation in HBsAg positive patients, especially those with high viral loads who may have a greater risk of HBV reactivation. This case is also the first report of HBV reactivation-related death during antiviral treatment with a new generation anti-HBV nucleoside analog, entecavir, that has both greater potential to suppress HBV replication and a high barrier to viral resistance mutations.[25,26] Therefore, if a potentially immune-enhancing drug is used for HBsAg positive cancer patients with high HBV DNA levels, it may be necessary to reduce the HBV DNA level as much as possible by antiviral treatment in advance, before initiating systemic chemotherapy.

Why did liver damage not occur, even when a very high HBV DNA level was detected after initial chemotherapy? Again, ATL patients usually have severe cellular immunodeficiency and a diagnosis of ATL is often made with the onset of opportunistic infections, such as pneumocystis pneumonia and cytomegalovirus infection.[27,28] On the other hand, the immune response, especially the HBV-specific T-cell response, is thought to be largely responsible for the onset of hepatitis and viral clearance during HBV infection.[29,30] Therefore, we speculate that his impaired cellular immune response could have allowed replication of the virus but could not attack the HBV-infected hepatocytes and induce hepatitis B just after the THP-COP regimen, suggesting that his immune response against HBV was weak before mogamulizumab treatment. One of the reasons may be limited virus replication due to the absence of precore and/or basal core promoter mutations that are associated with enhanced virus replication in patients with fulminant hepatitis B.[31,32]

What is the clinical significance of the HBsAg escape mutation in HBV reactivation? Retrospective analysis using his preserved sample after HBV reactivation also showed that he had a high titer of anti-HBs (110.1 mIU/mL, Fig. 1) and a common escape mutation (G145K) in the viral S region. Most HBV reactivation occurs after the decrease and disappearance of anti-HBs in patients initially seropositive for anti-HBs.[33] Escape mutations in HBsAg have been reported to be associated with sustained titers of anti-HBs during HBV reactivation,[34] which indicates that regular monitoring of anti-HBs titers may be insufficient to predict adequately HBV reactivation in such patients.

In summary, we first reported the sudden onset of acute liver failure, and death due to HBV reactivation, in an ATL patient who received anti-CCR4 antibody mogamulizumab monotherapy, despite antiviral prophylaxis with entecavir. This clinical course may not only offer important suggestions to prevent critical HBV reactivation in HBsAg positive cancer patients who receive immune-enhancing drugs such as anti-CCR4 antibody, but also provide a clue to understanding the pathogenesis of HBV reactivation following systemic chemotherapy.

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REFERENCES

1 Yeo W, Chan PK, Ho WM *et al.* Lamivudine for the prevention of hepatitis B virus reactivation in hepatitis B s-antigen seropositive cancer patients undergoing cytotoxic chemotherapy. *J Clin Oncol* 2004; **22**: 927–34.
2 Umemura T, Tanaka E, Kiyosawa K, Kumada H. Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. *Clin Infect Dis* 2008; **47**: e52–6.
3 Kusumoto S, Tanaka Y, Mizokami M, Ueda R. Reactivation of hepatitis B virus following systemic chemotherapy for malignant lymphoma. *Int J Hematol* 2009; **90**: 13–23.

4 Evens AM, Jovanovic BD, Su YC *et al.* Rituximab-associated hepatitis B virus (HBV) reactivation in lymphoproliferative diseases: meta-analysis and examination of FDA safety reports. *Ann Oncol* 2011; **22**: 1170–80.
5 Lok AS, Liang RH, Chiu EK, Wong KL, Chan TK, Todd D. Reactivation of hepatitis B virus replication in patients receiving cytotoxic therapy. Report of a prospective study. *Gastroenterology* 1991; **100**: 182–8.
6 Yeo W, Chan PK, Hui P *et al.* Hepatitis B virus reactivation in breast cancer patients receiving cytotoxic chemotherapy: a prospective study. *J Med Virol* 2003; **70**: 553–61.
7 Dervite I, Hober D, Morel P. Acute hepatitis B in a patient with antibodies to hepatitis B surface antigen who was receiving rituximab. *N Engl J Med* 2001; **344**: 68–9.
8 Hui CK, Cheung WW, Zhang HY *et al.* Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 2006; **131**: 59–68.
9 Yeo W, Chan TC, Leung NW *et al.* Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. *J Clin Oncol* 2009; **27**: 605–11.
10 Drafting Committee for the Management of Hepatitis B Virus Infection and the Japan Society of Hepatology. JSH Guidelines for the Management of Hepatitis B Virus Infection. *Hepatol Res* 2014; 44(Suppl S1): 1–58.
11 European Association For The Study Of The Liver *et al.* EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167–85.
12 Di Bisceglie AM, Lok AS, Martin P, Terrault N, Perrillo RP, Hoofnagle JH. Recent FDA warnings on hepatitis B reactivation with immune-suppressing and anti-cancer drugs: Just the tip of the iceberg? *Hepatology* 2014; **61**: 703–11.
13 Reddy KR, Beavers KL, Hammond SP, Lim JK, Falck-Ytter YT. American gastroenterological association institute guideline on the prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. *Gastroenterology* 2015; **148**: 215–9.
14 Ishida T, Utsunomiya A, Iida S *et al.* Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. *Clin Cancer Res* 2003; **9**: 3625–34.
15 Ishii T, Ishida T, Utsunomiya A *et al.* Defucosylated humanized anti-CCR4 monoclonal antibody KW-0761 as a novel immunotherapeutic agent for adult T-cell leukemia/lymphoma. *Clin Cancer Res* 2010; **16**: 1520–31.
16 Yamamoto K, Utsunomiya A, Tobinai K *et al.* Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. *J Clin Oncol* 2010; **28**: 1591–8.
17 Ishida T, Ueda R. Antibody therapy for Adult T-cell leukemia-lymphoma. *Int J Hematol* 2011; **94**: 443–52.
18 Ishida T, Joh T, Uike N *et al.* Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia-lymphoma: a multicenter phase II study. *J Clin Oncol* 2012; **30**: 837–42.
19 Mochida S, Takikawa Y, Nakayama N *et al.* Diagnostic criteria of acute liver failure: A report by the Intractable Hepato-Biliary Diseases Study Group of Japan. *Hepatol Res* 2011; **41**: 805–12.
20 Ishida T, Ueda R. Immunopathogenesis of lymphoma: focus on CCR4. *Cancer Sci* 2011; **102**: 44–50.
21 Ogura M, Ishida T, Hatake K *et al.* Multicenter phase II study of mogamulizumab (KW-0761), a defucosylated anti-cc chemokine receptor 4 antibody, in patients with relapsed peripheral T-cell lymphoma and cutaneous T-cell lymphoma. *J Clin Oncol* 2014; **32**: 1157–63.
22 Ishida T, Ito A, Sato F *et al.* Stevens-Johnson Syndrome associated with mogamulizumab treatment of adult T-cell leukemia / lymphoma. *Cancer Sci* 2013; **104**: 647–50.
23 Yano H, Ishida T, Inagaki A *et al.* Regulatory T-cell function of adult T-cell leukemia/lymphoma cells. *Int J Cancer* 2007; **120**: 2052–7.
24 Huang H, Li X, Zhu J *et al.* Entecavir vs lamivudine for prevention of hepatitis B virus reactivation among patients with untreated diffuse large B-cell lymphoma receiving R-CHOP chemotherapy: a randomized clinical trial. *JAMA* 2014; **312**: 2521–30.
25 Chang TT, Gish RG, de Man R *et al.* A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001–10.
26 Kim SJ, Hsu C, Song YQ *et al.* Hepatitis B virus reactivation in B-cell lymphoma patients treated with rituximab: analysis from the Asia Lymphoma Study Group. *Eur J Cancer* 2013; **49**: 3486–96.
27 Tashiro T, Yamasaki T, Nagai H, Kikuchi H, Nasu M. Immunological studies on opportunistic infection and the development of adult T-cell leukemia. *Intern Med* 1992; **31**: 1132–6.
28 Eizuru Y, Tamura K, Minamishima Y *et al.* Cytomegalovirus infections in adult T-cell leukemia patients. *J Med Virol* 1987; **23**: 123–33.
29 Chisari FV. Rous-Whipple Award Lecture. Viruses, immunity, and cancer: lessons from hepatitis B. *Am J Pathol* 2000; **156**: 1117–32.
30 Isogawa M, Tanaka Y. Immunobiology of hepatitis B virus infection. *Hepatol Res* 2015; **45**: 179–89.
31 Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. *N Engl J Med* 1991; **324**: 1699–704.
32 Ozasa A, Tanaka Y, Orito E *et al.* Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. *Hepatology* 2006; **44**: 326–34.
33 Pei SN, Ma MC, Wang MC *et al.* Analysis of hepatitis B surface antibody titers in B cell lymphoma patients after rituximab therapy. *Ann Hematol* 2012; **91**: 1007–12.
34 Westhoff TH, Jochimsen F, Schmittel A *et al.* Fatal hepatitis B virus reactivation by an escape mutant following rituximab therapy. *Blood* 2003; **102**: 1930.

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ORIGINAL ARTICLE

Reactivation of hepatitis B virus (HBV) infection in adult T-cell leukemia–lymphoma patients with resolved HBV infection following systemic chemotherapy

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Abstract Reactivation of hepatitis B virus (HBV) infection may occur in adult T-cell leukemia–lymphoma (ATL) patients with resolved HBV infection who receive monotherapy with the anti-CC chemokine receptor 4 monoclonal antibody, mogamulizumab. However, there is little evidence regarding the incidence and characteristics of HBV reactivation in ATL patients receiving systemic chemotherapy, including the use of this antibody. We conducted a retrospective study for 24 ATL patients with resolved HBV infection underwent regular HBV DNA monitoring to assess HBV reactivation in Nagoya City University Hospital between January 2005 and June 2013. With median HBV DNA follow-up of 238 days (range 57–1420), HBV reactivation (defined as the detection of HBV DNA) was observed in three (12.5 %) of 24 patients with resolved HBV infection. No hepatitis due to HBV reactivation occurred in those patients who were diagnosed with HBV DNA levels below 2.1 log copies/mL and who received antiviral drugs. Mogamulizumab was administered prior to HBV reactivation in two of three HBV-reactivated patients. In the mogamulizumab era, further well-designed prospective studies are warranted to estimate the incidence of HBV reactivation and to establish regular HBV DNA monitoring-guided preemptive antiviral therapy for such patients.

Keywords Reactivation · HBV · CCR4 · Mogamulizumab · ATL

Abbreviations

HBV	Hepatitis B virus
ATL	Adult T-cell leukemia–lymphoma
HBsAg	Hepatitis B surface antigen
Anti-HBc	Antibodies against hepatitis B core antigen
Anti-HBs	Antibodies against hepatitis B surface antigen
CCR4	CC chemokine receptor 4

Introduction

Reactivation of hepatitis B virus (HBV) infection has been reported as a potentially fatal complication of systemic chemotherapy [1–6]. HBV reactivation may occur not only in hepatitis B surface antigen (HBsAg)-positive patients, but also in patients with resolved HBV infection who are seronegative for HBsAg but seropositive for antibodies

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against hepatitis B core antigen (anti-HBc) and/or antibodies against HBsAg (anti-HBs).

Chemotherapy containing the anti-CD20 monoclonal antibody, rituximab plus steroids has been shown to be an important risk factor for HBV reactivation in B-cell lymphoma patients with resolved HBV infection [2, 3]. Recently, the anti-CC chemokine receptor 4 (CCR4) monoclonal antibody, mogamulizumab, was developed and introduced into the management of adult T-cell leukemia–lymphoma (ATL) [7–12]. A dose-finding study showed that mogamulizumab monotherapy could induce HBV reactivation-related hepatitis in an ATL patient with resolved HBV infection [9, 13].

However, there is little evidence regarding the incidence and characteristics of HBV reactivation in ATL patients with resolved HBV infection who were receiving systemic chemotherapy including this antibody. We conducted here a retrospective study in a single institution to evaluate the risk of HBV reactivation in these patients who underwent regular monitoring of HBV DNA levels during and after chemotherapy.

Patients and methods

Between January 2005 and June 2013, 66 patients were diagnosed with ATL in Nagoya City University Hospital. Baseline serological markers for HBsAg, anti-HBc, and anti-HBs were measured to evaluate their viral status before systemic chemotherapy. Antiviral prophylaxis was provided to the HBsAg-positive patients before the initiation of systemic chemotherapy. HBV DNA levels were assessed in HBsAg-negative patients who were seropositive for anti-HBc and/or anti-HBs. Patients seronegative for HBsAg but with detectable of HBV DNA were considered to have occult HBV infection, and antiviral prophylaxis was provided to those patients. HBsAg-negative patients seropositive for anti-HBc and/or anti-HBs but without detectable of HBV DNA were considered to have resolved HBV infection and their HBV DNA levels were monitored regularly (monthly in principle) for HBV DNA levels during chemotherapy and at least 1 year after chemotherapy; HBV reactivation was defined as the detection of HBV DNA. If HBV reactivation was confirmed, antiviral drugs were given immediately (preemptive antiviral therapy).

All baseline serological markers of HBsAg, anti-HBc and anti-HBs were measured by the laboratory in this hospital, using the following methods and cut-off values: CLEIA with cut-off values for HBsAg, anti-HBc and anti-HBs were 1.0 C.O.I, 1.0 INH % and 10.0 mIU/mL, respectively, from January 2005 to December 2010, CLEIA with cut-off values for HBsAg, anti-HBc and ant-HBs were 0.03 mIU/mL, 1.0 C.O.I, and 10.0 mIU/mL, respectively, from January 2011.

HBV DNA levels were measured by an outside laboratory (SRL, Inc.; Tokyo, Japan) or by the laboratory in this hospital, using the following methods and cut-off values: transcription-mediated amplification test with a cut-off value of 3.7 LGE/mL from January 2005 to April 2006, Amplicor HBV monitor test with a cut-off value of 2.6 log copies/mL from April 2006 to May 2008, COBAS AmpliPrep/COBAS TaqMan HBV test (v1.0) with a cut-off value of 1.8 log copies/mL from May 2008 to July 2009, and COBAS AmpliPrep/COBAS TaqMan HBV test (v2.0) with a cut-off value of 2.1 log copies/mL from July 2009.

For the analysis of HBV sequences, nucleic acids were extracted from the preserved serum specimens (200 μL) and subjected to PCR to amplify HBV genomes within the short S region [nucleotides (nt) 427–607] and the basal core promoter (BCP)/precore (PC) regions [nt 1628–2047] followed by direct sequencing using the ABI Prism Big Dye ver. 3.1 kit in an ABI 3100 DNA automated sequencer (Applied Biosystems, Foster City, CA). HBV genotypes were determined by molecular evolutionary analysis [14].

To compare the baseline characteristics and ATL treatment of the patients with and without HBV reactivation, we used the Chi-square test and two-sided Fisher's exact test for categorical data, and the Mann–Whitney U test for continuous variables. A two-tailed p value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS (version 22.0) statistical software for Windows, using data fixed on August 31, 2013. This study was approved by the Institutional Review Board of Nagoya City University. All patients gave written informed consent.

Results

The status of HBV infection at baseline was as follows (Fig. 1): HBsAg-positive (n = 2, 3.0 %), HBsAg-negative (n = 63, 95.5 %), and no serological HBV assessment (n = 1, 1.5 %). Of the 63 HBsAg-negative patients, 31 (49.2 %) were anti-HBc positive and/or anti-HBs positive. Of the remaining 32 patients, 31 were anti-HBc negative and anti-HBs negative, and one had no data for anti-HBc and anti-HBs. Because HBV DNA below 1.8 log copies/mL was detected at baseline in one patient who was anti-HBc positive and anti-HBs positive at baseline (and who was therefore judged to have occult HBV infection), antiviral drugs were administered before initiating systemic chemotherapy. Finally, 24 of 31 patients with resolved HBV infection underwent regular HBV DNA monitoring (Fig. 1). For these 24 ATL patients, initial systemic chemotherapy included the following regimens: CHOP (n = 7, 29.2 %), VCAP-AMP-VECP (n = 13, 54.2 %) and others (n = 4, 16.6 %) (Table 1). Systemic chemotherapy was started in 6

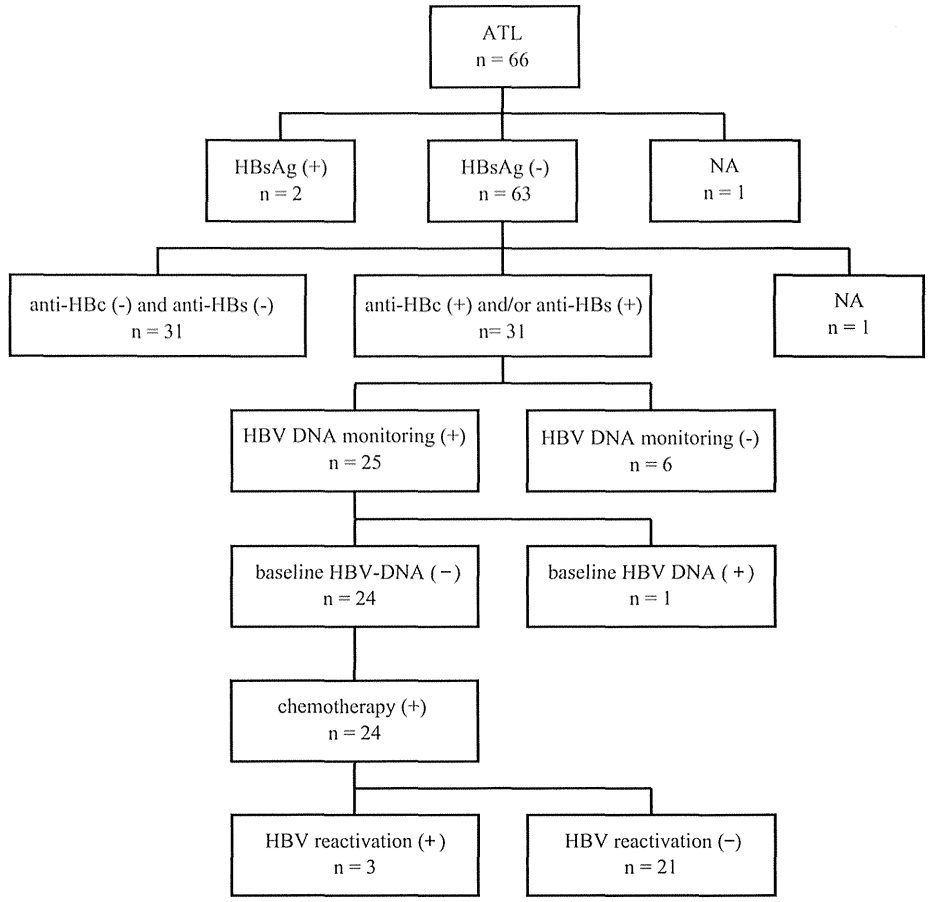

Fig. 1 Baseline serological markers of HBV infection in the 66 ATL patients. Two patients were HBsAg-positive, 63 were HBsAg-negative, the last was not available for serological HBV assessment. Of the 63 HBsAg-negative patients, 31 were anti-HBc-positive and/or anti-HBs-positive. One patient had detectable HBV DNA at baseline, and was judged as having occult HBV infection. Regular HBV DNA monitoring was performed in 24 of 31 patients with resolved HBV infection and 3 patients suffered HBV reactivation. *HBV* hepatitis B virus, *ATL* adult T-cell leukemia–lymphoma, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibodies against hepatitis B core antigen, *anti-HBs* antibodies against hepatitis B surface antigen, *NA* not available

patients before HBV DNA monitoring. For the 24 patients with resolved HBV infection during and after systemic chemotherapy, regular monitoring of HBV DNA was conducted with a median interval of 30 days (range 2–703).

HBV reactivation was observed in 3 (12.5 %) of 24 patients with resolved HBV infection, with a median HBV DNA follow-up of 238 days (range 57–1420). No hepatitis due to HBV reactivation occurred in those patients who were diagnosed with HBV DNA levels below 2.1 log copies/mL and who received antiviral drugs (entecavir, 0.5 mg/day), resulting in no detectable HBV DNA levels during antiviral treatment.

There was no statistically significant difference in baseline characteristics and ATL treatment between patients with and without reactivation in this retrospective analysis (Table 1). The characteristics of 3 patients with HBV reactivation are shown in Table 2; all were male, and seropositive for anti-HBc and anti-HBs at baseline, and received the VCAP-AMP-VECP regimen as initial treatment. Mogamulizumab was administered prior to HBV reactivation in 2 of 3 HBV-reactivated patients. The anti-HBs titers of 3 patients decreased at reactivation compared to baseline titers in 3 patients. Their HBV genotypes were determined as C. HBV mutations were not found in the precore region or basal core promoter. One patient died due to ATL progression.

The clinical course of case 1 is shown in Fig. 2. HBV reactivation was confirmed with HBV DNA levels below 2.1 log copies/mL, 3 months after initiating mogamulizumab-containing chemotherapy as initial treatment for ATL. The patient presented with elevation of transaminase levels after detection of HBV DNA, it considered not viral hepatitis, but drug-induced liver damage because of transient and slight increase of HBV DNA levels. Reemergence of HBV was observed repeatedly after withdrawal of antiviral drugs following the development of drug-induced allergic rash or interstitial pneumonia. The patient maintains complete remission of ATL with undetectable of HBV DNA after withdrawal of antiviral drugs over 3 years after mogamulizumab-containing chemotherapy.

Discussion

This study showed that the incidence of HBV reactivation among ATL patients with resolved HBV infection who received systemic chemotherapy was 12.5 %. Preemptive antiviral therapy, guided by regular HBV DNA monitoring,

Table 1 Baseline characteristics and treatment of 24 ATL patients with resolved HBV infection who underwent HBV DNA monitoring following systemic chemotherapy

	HBV reactivation (+) $n = 3$	HBV reactivation (−) $n = 21$	p value
Median age (range)	59 (58–65)	64 (41–77)	0.822
Sex			0.217
Male	3	9	
Female	0	12	
ATL type of disease			0.090
Acute	1	17	
Lymphoma	2	1	
Chronic	0	2	
Smoldering	0	1	
ECOG performance status			0.530
0 or 1	3	14	
2 or more	0	7	
Baseline HBV status			1.00
Anti-HBc positive and anti-HBs positive	3	18	
Anti-HBc positive and anti-HBs negative	0	3	
Anti-HBc negative and anti-HBs positive	0	0	
Baseline anti-HBs titers (mIU/mL)			0.728
<10	0	3	
≥10, <100	2	8	
≥100	1	10	
Initial chemotherapy regimen[a]			0.396
CHOP	0	7	
VCAP-AMP-VECP	3	10	
Others	0	4	
Mogamulizumab administration[b]			0.576
(+)	2	9	
(−)	1	12	
Allogeneic HSCT[c]			1.00
(+)	1	5	
(−)	2	16	
Year enrolled for HBV DNA monitoring			–
2005–2006	0	0	
2006–2008	0	4	
2008–2009	0	3	
2009–2013	3	14	
Median HBV DNA follow-up time (range)[d]	640 (637–1030)	227 (57–1420)	–

HBV hepatitis B virus, *ATL* adult T-cell leukemia–lymphoma, *ECOG* Eastern Cooperative Oncology Group, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibodies against hepatitis B core antigen, *anti-HBs* antibodies against hepatitis B surface antigen, *CHOP* cyclophosphamide, doxorubicin, vincristine, prednisolone, *VCAP-AMP-VECP* VCAP (vincristine, cyclophosphamide, doxorubicin, prednisolone)-AMP (doxorubicin, ranimustine, prednisolone)-VECP (vindesine, etoposide, carboplatin, prednisolone), *HSCT* hematopoietic stem cell transplantation

[a] Initial chemotherapy regimen for adult T-cell leukemia–lymphoma was given during HBV DNA monitoring

[b] In 2 of 3 HBV-reactivated cases, mogamulizumab was given prior to HBV reactivation

[c] One patient received allogeneic hematopoietic stem transplantation after HBV reactivation

[d] HBV DNA follow-up time indicates the time from the date of baseline HBV DNA measurement until the date of the last HBV DNA measurement

was effective in preventing hepatitis due to HBV reactivation in all three patients. Most of HBV reactivation has been reported to occur in B-cell lymphoma, especially in those who received rituximab-containing chemotherapy [2–4, 6]. This is the first report regarding the risk of HBV reactivation focused on ATL patients with resolved HBV infection, which suggesting that the risk of HBV reactivation in ATL patients may be similar to that in B-cell lymphoma patients [15, 16].

ATL is a mature T-cell lymphoma and human T-cell leukemia virus type-1 plays a role in its pathogenesis. Aggressive ATL has been reported to have a poor prognosis with a median overall survival of approximately 1 year, regardless of intensive chemotherapy [17]. The anti-CCR4 monoclonal antibody, mogamulizumab has been shown recently to be effective and safe for aggressive ATL patients in the setting of monotherapy or combined with conventional chemotherapy [9, 11, 18]. It is expected that mogamulizumab will enable long-term disease control, so more HBV reactivation events may be predicted because CCR4 is a chemokine receptor expressed on T-helper type 2 and regulatory T cells [7, 19], and is thought to have an important

Table 2 Characteristics of 3 patients with HBV reactivation

	Case 1	Case 2	Case 3
Age	65	59	58
Sex	Male	Male	Male
Type of ATL	Lymphoma	Lymphoma	Acute
ECOG performance status	1	1	0
Baseline HBV status			
HBsAg	(−)	(−)	(−)
Anti-HBc titers	98.1 %	3.6 C.O.I	1.5 C.O.I
Anti-HBs titers	20.0 mIU/mL	24.0 mIU/mL	>1000.0 mIU/mL
HBV DNA levels	Not detectable	Not detectable	Not detectable
Chemotherapy regimens before HBV reactivation			
	VCAP-AMP-VECP plus mogamulizumab	VCAP-AMP-VECP	VCAP-AMP-VECP Mogamulizumab CHOP DeVIC etc.
Number of regimens	1	1	7
Allogeneic HSCT[a]	No	Yes	No
After HBV reactivation			
Time to reactivation (day)[b]	90	71	541
HBV DNA levels at reactivation (log copies/mL)	<2.1	<2.1	<2.1
Peak HBV DNA levels (log copies/mL)	2.3	<2.1	<2.1
Anti-HBs titers	17.6 mIU/mL	22.0 mIU/mL	566.5 mIU/mL
HBV genotype	C	C	C
HBV mutation of precore region or basal core promoter	Wild	Wild	NA
Antiviral drugs	Entecavir, lamivudine	Entecavir	Entecavir
Hepatitis due to HBV reactivation	No	No	No
HBV DNA follow-up time (day)[c]	1030	640	637
Outcome	Alive (CR1)	Alive (CR1)	Death due to ATL progression

HBV hepatitis B virus, *ATL* adult T-cell leukemia–lymphoma, *ECOG* Eastern Cooperative Oncology Group, *HBsAg* hepatitis B surface antigen, *anti-HBc* antibodies against hepatitis B core antigen, *anti-HBs* antibodies against hepatitis B surface antigen, *VCAP-AMP-VECP* VCAP (vincristine, cyclophosphamide, doxorubicin, prednisolone)-AMP (doxorubicin, ranimustine, prednisolone)-VECP (vindesine, etoposide, carboplatin, prednisolone), *CHOP* cyclophosphamide, doxorubicin, vincristine, prednisolone, *DeVIC* dexamethasone, etoposide, ifosfamide, carboplatin, *CR1* first complete response

[a] One patient (case 2) received allogeneic hematopoietic stem transplantation after HBV reactivation

[b] Time to reactivation indicates the time from the date of baseline HBV DNA measurement until the date of the confirmation of HBV reactivation

[c] HBV DNA follow-up time indicates the time from the date of baseline HBV DNA measurement until the date of the last HBV DNA measurement

role in maintaining the balance of the human immune system. The mechanism whereby mogamulizumab causes HBV reactivation is not fully understood; a reduction of numbers of CCR4-expressing cells following this antibody treatment might be associated with an imbalance of antiviral immunity, resulting in the development of HBV reactivation [9, 13]. Although HBV reactivation was confirmed in 2 of 11 patients who received mogamulizumab, this study did not prove that HBV reactivation is associated with mogamulizumab therapy, partly because of the small sample size.

This study has the following limitations: a retrospective study in a single institution with a small sample size, and the diagnosis of HBV reactivation at early stage when only when HBV DNA became detectable (below 2.1 log copies/mL) by PCR. Because antiviral treatments after the onset of hepatitis are often insufficient to control HBV reactivation, preemptive antiviral therapy guided by regular HBV DNA monitoring, whereby the antiviral drug is given immediately when HBV DNA becomes detectable, is recommended by some guidelines to prevent hepatitis due to HBV reactivation [20, 21]. However, the definition of HBV reactivation and cut-off values of HBV DNA levels, along with the timing of initiation of antiviral treatment in patients with resolved HBV infection, have not been fully investigated yet.

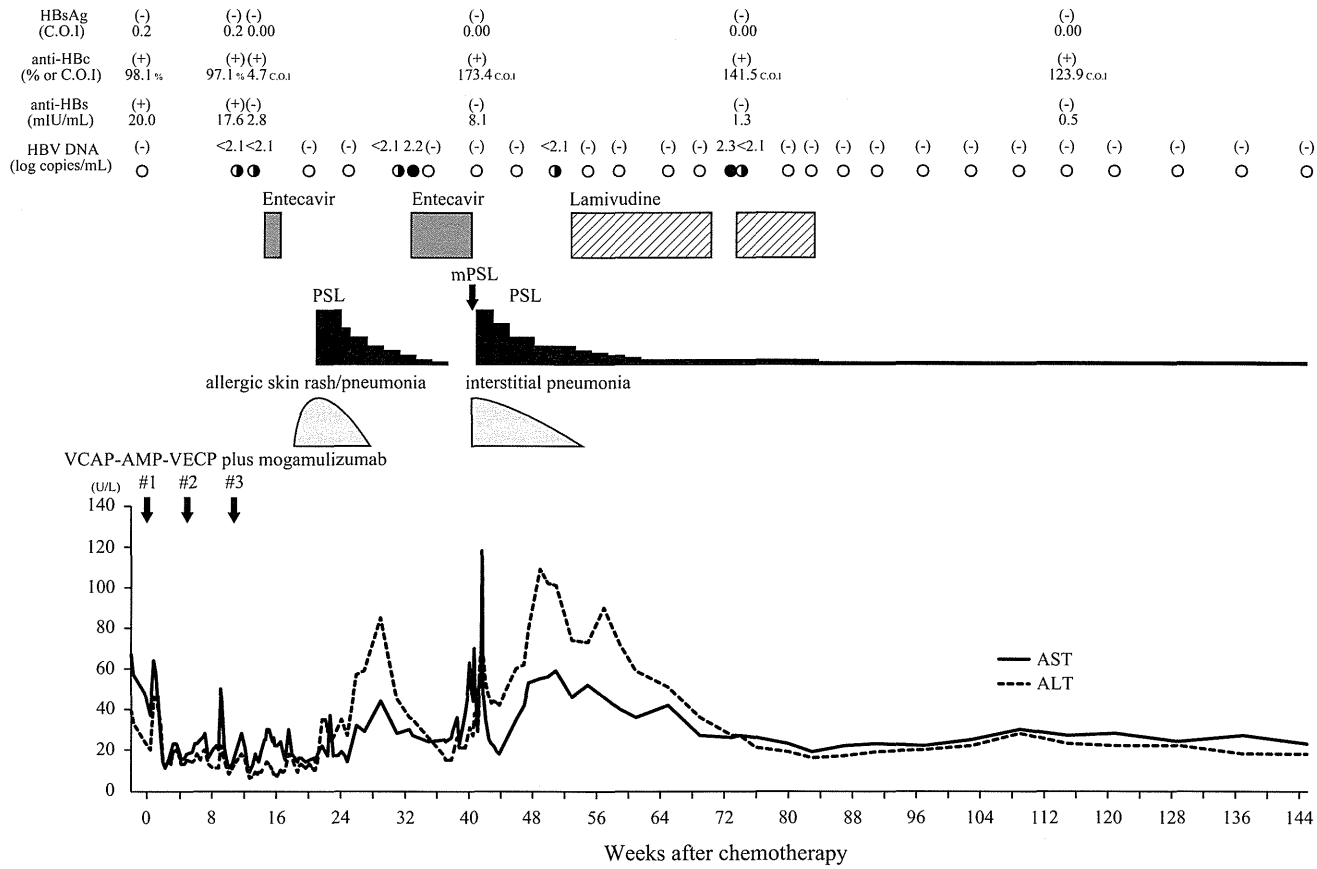

Fig. 2 Clinical course of case 1. A 65-year-old male was diagnosed as having adult T-cell leukemia–lymphoma of lymphoma type and received VCAP-AMP-VECP plus mogamulizumab combined chemotherapy. At 3 months after systemic chemotherapy, HBV reactivation was confirmed with HBV DNA levels below 2.1 log copies/mL and antiviral therapy (entecavir, 0.5 mg/day) was given immediately with no HBV-related hepatitis. He presented with elevation of transaminase levels after detection of HBV DNA, it considered not viral hepatitis but drug-induced liver damage because of transient and slight increase of HBV DNA levels. Because he suffered from an allergic rash and interstitial pneumonia (IP), entecavir could not be continued. Consequently, reemergence of HBV (HBV DNA levels of 2.2 log copies/mL) was observed at 3 months after the first detection of HBV reactivation. However, he discontinued entecavir because of the occurrence of IP, and HBV reactivation was again observed. Lamivudine was given for HBV reactivation, but was discontinued due to mild renal dysfunction, which resulted again in replication of HBV (HBV DNA levels of 2.3 log copies/mL) at 18 months after initiating mogamulizumab-containing chemotherapy. The *open circles* show undetectable PCR signals during HBV DNA monitoring; the *half-filled circles* show PCR signals indicating HBV DNA levels below 2.1 log copies/mL; and the *filled circles* show detectable PCR signals indicating HBV DNA levels of 2.1 log copies/mL or more. *HBV* hepatitis B virus, *PSL* prednisolone, *mPSL* methylprednisolone, *AST* aspartate transaminase, *ALT* alanine aminotransferase, *VCAP-AMP-VECP* VCAP (vincristine, cyclophosphamide, doxorubicin, prednisolone)-AMP (doxorubicin, ranimustine, prednisolone)-VECP (vindesine, etoposide, carboplatin, prednisolone)

In conclusion, the incidence of HBV reactivation was 12.5 % in ATL patients with resolved HBV infection following systemic chemotherapy. In mogamulizumab era, further well-designed prospective studies are warranted to estimate the incidence of HBV reactivation and to establish regular HBV DNA monitoring-guided preemptive antiviral therapy for these patients.

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References

1. Lok AS, Liang RH, Chiu EK, Wong KL, Chan TK, Todd D. Reactivation of hepatitis B virus replication in patients receiving cytotoxic therapy. Report of a prospective study. Gastroenterology. 1991;100(1):182–8.
2. Hui CK, Cheung WW, Zhang HY, Au WY, Yueng YH, Leung AY, et al. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. Gastroenterology. 2006;131(1):59–68.
3. Yeo W, Chan TC, Leung NW, Lam WY, Mo FK, Chu MT, et al. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. J Clin Oncol. 2009;27(4):605–11 (Epub 2008/12/17).
4. Kusumoto S, Tanaka Y, Mizokami M, Ueda R. Reactivation of hepatitis B virus following systemic chemotherapy for malignant lymphoma. Int J Hematol. 2009;90(1):13–23 (Epub 2009/06/23).
5. Kusumoto S, Tanaka Y, Ueda R, Mizokami M. Reactivation of hepatitis B virus following rituximab-plus-steroid combination chemotherapy. J Gastroenterol. 2011;46(1):9–16 (Epub 2010/10/07).
6. Mitka M. FDA: increased HBV reactivation risk with ofatumumab or rituximab. JAMA. 2013;310(16):1664.
7. Ishida T, Utsunomiya A, Iida S, Inagaki H, Takatsuka Y, Kusumoto S, et al. Clinical significance of CCR4 expression in adult T-cell leukemia/lymphoma: its close association with skin involvement and unfavorable outcome. Clin Cancer Res. 2003;9(10 Pt 1):3625–34.
8. Ishii T, Ishida T, Utsunomiya A, Inagaki A, Yano H, Komatsu H, et al. Defucosylated humanized anti-CCR4 monoclonal antibody KW-0761 as a novel immunotherapeutic agent for adult T-cell leukemia/lymphoma. Clin Cancer Res. 2010;16(5):1520–31.
9. Yamamoto K, Utsunomiya A, Tobinai K, Tsukasaki K, Uike N, Uozumi K, et al. Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia–lymphoma and peripheral T-cell lymphoma. J Clin Oncol. 2010;28(9):1591–8.
10. Ishida T, Ueda R. Antibody therapy for Adult T-cell leukemia–lymphoma. Int J Hematol. 2011;94(5):443–52.
11. Ishida T, Joh T, Uike N, Yamamoto K, Utsunomiya A, Yoshida S, et al. Defucosylated anti-CCR4 monoclonal antibody (KW-0761) for relapsed adult T-cell leukemia–lymphoma: a multicenter phase II study. J Clin Oncol. 2012;30(8):837–42.
12. Motohashi K, Suzuki T, Kishimoto K, Numata A, Nakajima Y, Tachibana T, et al. Successful treatment of a patient with adult T cell leukemia/lymphoma using anti-CC chemokine receptor 4 monoclonal antibody mogamulizumab followed by allogeneic hematopoietic stem cell transplantation. Int J Hematol. 2013;98(2):258–60.
13. Nakano N, Kusumoto S, Tanaka Y, Ishida T, Takeuchi S, Takatsuka Y, et al. Reactivation of hepatitis B virus in a patient with adult T-cell leukemia–lymphoma receiving the anti-CC chemokine receptor 4 antibody mogamulizumab. Hepatol Res. 2014;44(3):354–7.
14. Shin IT, Tanaka Y, Tateno Y, Mizokami M. Development and public release of a comprehensive hepatitis virus database. Hepatol Res. 2008;38(3):234–43.
15. Hsu C, Tsou HH, Lin SJ, Wang MC, Yao M, Hwang WL, et al. Chemotherapy-induced hepatitis B reactivation in lymphoma patients with resolved HBV infection: a prospective study. Hepatology. 2014;59(6):2092–100.
16. Kusumoto S, Tanaka Y, Suzuki R, Watanabe T, Nakata M, Takasaki H, et al. Prospective nationwide observational study of hepatitis B virus (HBV) DNA monitoring and preemptive antiviral therapy for HBV reactivation in patients with B-cell non-Hodgkin lymphoma following rituximab containing chemotherapy: results of interim analysis. Blood. 2012; 120(21):abstract 2641.
17. Tsukasaki K, Utsunomiya A, Fukuda H, Shibata T, Fukushima T, Takatsuka Y, et al. VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia–lymphoma: Japan Clinical Oncology Group Study JCOG9801. J Clin Oncol. 2007;25(34):5458–64.
18. Jo T, Ishida T, Takemoto S, Suzushima H, Uozumi K, Yamamoto K, et al. Randomized phase II study of mogamulizumab (KW-0761) plus VCAP-AMP-VECP (mLSG15) versus mLSG15 alone for newly diagnosed aggressive adult T-cell leukemia–lymphoma (ATL). J Clin Oncol 2013; 31: (suppl; abstr 8506).
19. Ishida T, Ueda R. Immunopathogenesis of lymphoma: focus on CCR4. Cancer Sci. 2011;102(1):44–50.
20. Oketani M, Ido A, Uto H, Tsubouchi H. Prevention of hepatitis B virus reactivation in patients receiving immunosuppressive therapy or chemotherapy. Hepatol Res. 2012;42(7):627–36 Epub 2012/06/13.
21. EASL clinical practice guidelines. Management of chronic hepatitis B virus infection. J Hepatol. 2012;57(1):167–85 Epub 2012/03/23.

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IMAGES IN HEMATOLOGY

CD30-positive primary bone marrow lymphoma mimicking Hodgkin lymphoma

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An 89-year-old Japanese man was admitted to this hospital for persistent fever for over 1 month and pancytopenia (a white blood cell count of 1700 /μL with 46 % neutrophils, 33 % lymphocytes, 7 % atypical lymphocytes, and 14 % monocytes, a hemoglobin level of 8.4 g/dL, and a platelet count of 11.9 × 10^4 /μL). A lymphoid neoplasm was suspected because of his markedly elevated soluble interleukin-2 receptor level of 16,709 U/mL, but computed tomography revealed no lymphadenopathy or hepatosplenomegaly. An ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) scan showed disseminated FDG uptake only in the bone marrow (Fig. 1).

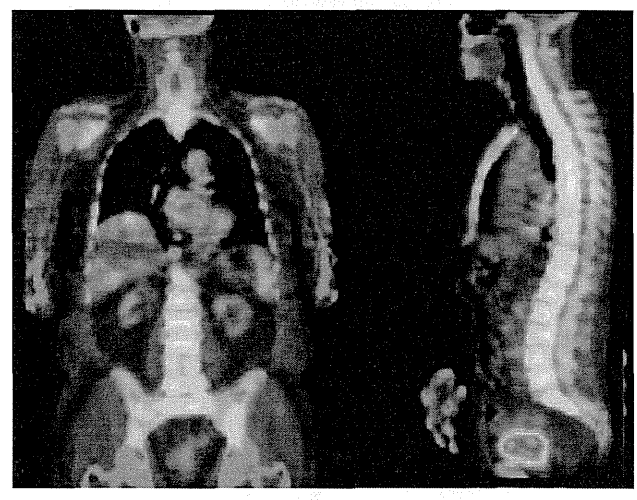

Fig. 1 This ^{18}F-fluorodeoxyglucose positron emission tomography scan shows disseminated FDG uptake in the bone marrow without evidence of the involvement of other organs. Focal weak FDG uptake in the liver and the spleen was considered to be within normal range

Specimens of bone marrow aspiration and trephine biopsy showed hypercellular bone marrow and large binucleated or multinucleated cells resembling Reed-Sternberg (RS) cells, which were found sparsely in a background of small- to intermediate-sized lymphocytes and activated histiocytes (Fig. 2a). Bone marrow fibrosis was also seen. Immunostaining was carried out for various markers, including CD3, CD4, CD8, CD15 (Fig. 2b), CD20 (Fig. 2c), CD30 (Fig. 2d), CD45 (Fig. 2e), CD56, PAX5 (Fig. 2f), ALK, granzymeB, and BOB.1 (Fig. 2g), and nuclear staining for Epstein-Barr virus encoded RNA was performed by in situ hybridization (EBER-ISH). RS-like cells were positive for CD30, CD45, PAX5 and BOB.1. More specifically, a subset of the tumor cells was positive for BOB.1 but negative for the other antigens and EBER-ISH. The lymphocytes around the tumor cells were predominantly CD8-positive T-cells.

Southern blot analysis of the bone marrow did not reveal clonal rearrangements of the joining heavy chain immunoglobulin. On the other hand, polymerase chain reaction (PCR) of immunoglobulin heavy chain gene locus using D_H and J_H consensus primers demonstrated a monoclonal

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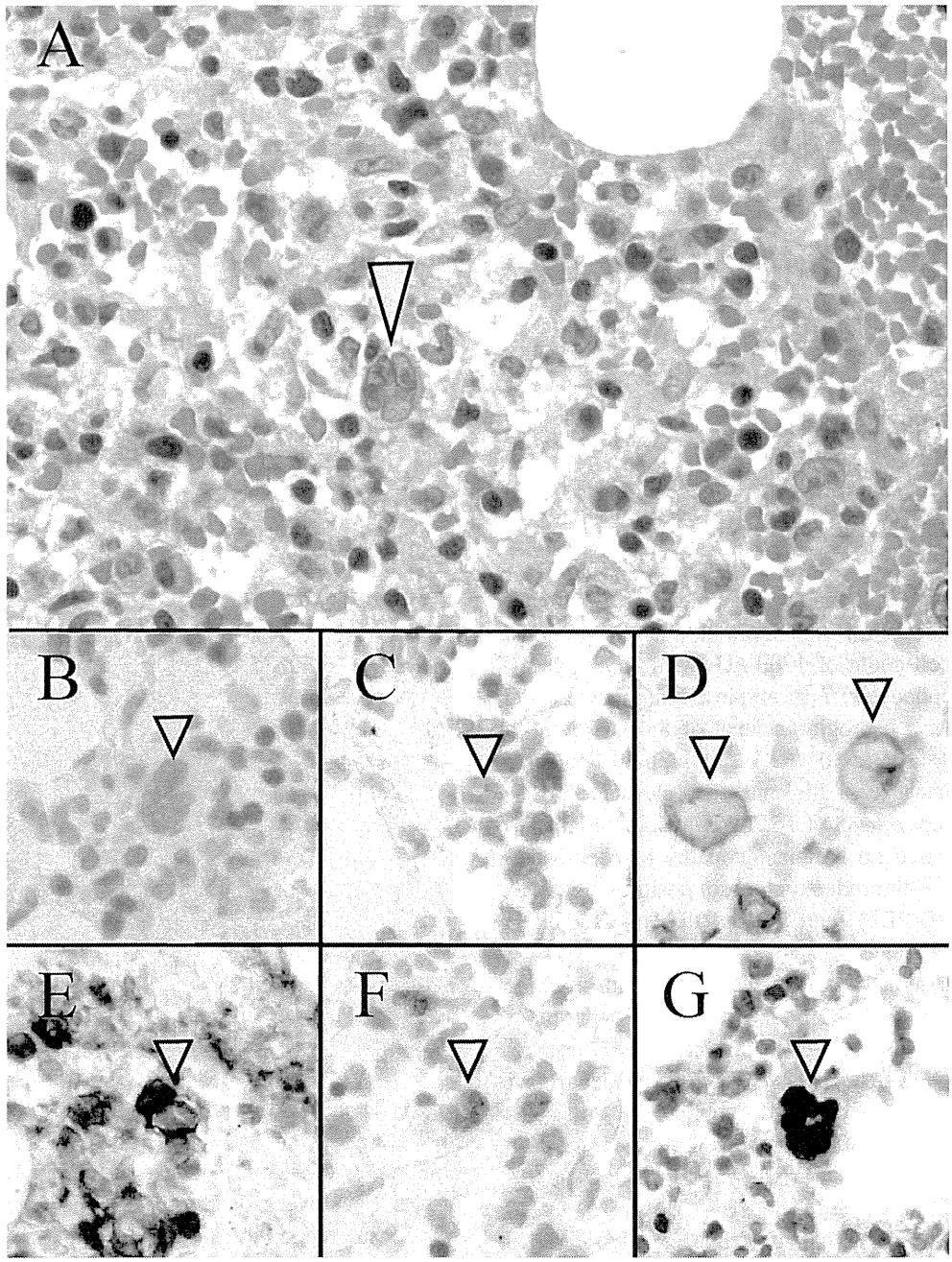

Fig. 2 Reed-Sternberg-like cells, indicated by *arrows*, are found sparsely in the bone marrow (**a** Hematoxylin and Eosin staining). These cells are negative for CD15 (**b**) and CD20 (**c**) but positive for CD30 (**d**), CD45 (**e**) and PAX5 (**f**). Some neoplastic cells are positive for BOB.1 (**g**). (Original magnification of all panels, ×400)

amplification product. Furthermore, cytogenetic study of the bone marrow showed an abnormal karyotype of 46,XY,del(3)(p?) in four of the 20 cells analyzed. Finally, the diagnosis of primary bone marrow large B cell lymphoma was established.

Descriptions of primary bone marrow lymphoma (PBML) in the literature have been limited, and its diagnostic criteria have not been established. Because our patient did not show any evidence of organ involvement, other than bone marrow assessed by CT and PET scan, the diagnosis of PBML was thought to be reasonable.

In theory, RS-like cells should be interpreted carefully because they are not specific for Hodgkin lymphoma (HL) and have been found not only in cases of other malignancies, such as non-HL, carcinomas and sarcomas, but also in reactive processes. In the current case, HL was ruled

out because the RS-like cells were negative for CD15 and EBER-ISH but positive for CD45 and BOB.1.

A further question regarding the current case was the origin of the tumor cells and this was thought to be B lymphocytes, based on the positivity for PAX5 immunostaining, clonal immunoglobulin heavy chain gene rearrangement detected by PCR analysis and negativity for NK/T-cell markers.

Conflict of interest The authors declare that they have no conflict of interest.

Immune-mediated antitumor effect by type 2 diabetes drug, metformin

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Metformin, a prescribed drug for type 2 diabetes, has been reported to have anti-cancer effects; however, the underlying mechanism is poorly understood. Here we show that this mechanism may be immune-mediated. Metformin enabled normal but not T-cell–deficient SCID mice to reject solid tumors. In addition, it increased the number of $CD8^+$ tumor-infiltrating lymphocytes (TILs) and protected them from apoptosis and exhaustion characterized by decreased production of IL-2, TNFα, and IFNγ. $CD8^+$ TILs capable of producing multiple cytokines were mainly PD-1^-Tim-3^+, an effector memory subset responsible for tumor rejection. Combined use of metformin and cancer vaccine improved $CD8^+$ TIL multifunctionality. The adoptive transfer of antigen-specific $CD8^+$ T cells treated with metformin concentrations as low as 10 μM showed efficient migration into tumors while maintaining multifunctionality in a manner sensitive to the AMP-activated protein kinase (AMPK) inhibitor compound C. Therefore, a direct effect of metformin on $CD8^+$ T cells is critical for protection against the inevitable functional exhaustion in the tumor microenvironment.

immune exhaustion | CD8T cells | antitumor immunity | tumor microenvironment | multifunctionality

In chronic infectious diseases and cancer, $CD8^+$ T cells specific for viral and/or tumor antigens undergo repeated TCR stimulation because of persistent pathogens or cancer cells and gradually lose their ability to secrete IL-2, TNFα, and IFNγ, eventually undergoing apoptotic elimination in a process known as immune exhaustion (1). This worsening immune function is accompanied by phenotypic changes in $CD8^+$ T cells, including the expression of exhaustion markers such as PD-1 and Tim-3 (2). Antitumor immunity is enhanced in mice deficient in PD-1 or its ligands PDL-1 and PDL-2 (2-4). Galectin 9, a Tim-3 ligand, is secreted by many tumor cells as well as by FoxP3-expressing regulatory T-cell (Treg) and inhibits Tim-3–expressing Th1 cells (5). An anti–Tim-3 antibody that blocks the galectin 9–Tim-3 pathway was found to accelerate antitumor immunity (6). Furthermore, the administration of blocking antibodies against both PD-1 and Tim-3 induced a more profound tumor rejection in comparison with that achieved with either antibody alone (7). The management of functional T-cell exhaustion within tumor tissues is currently an extensive focus in tumor immunotherapy (8, 9), together with efforts to neutralize immune-inhibitory Treg and myeloid-derived suppressor cell (MDSC).

Metformin (dimethylbiguanide) has been widely prescribed for type 2 diabetes. Its unique pharmacological features include its antihyperglycemic efficacy, which counters insulin resistance (10, 11). Early metformin use increases the survival of patients with obesity-involved type 2 diabetes and/or cardiovascular disease (12). In addition, recent reports have described the unexpected anticancer effects of metformin in patients with type 2 diabetes (13). Insulin-based diabetes treatment is associated with an increased cancer risk (14–17), whereas metformin use has been shown to decrease the frequency of specific cancers (18–21). Two independent metaanalyses of epidemiological studies concluded that compared with other treatments, metformin is associated with a 30–40% reduction in the incidence of cancer among patients with type 2 diabetes, indicating the need to investigate the anticancer mechanisms of metformin and conduct long-term randomized controlled trials (RCTs) (22, 23).

In the HER-2/*neu* transgenic mouse breast cancer model, metformin treatment decreased the tumor burden and was associated with an increased life span (24). Combined use of metformin with chemotherapeutic agents such as cisplatin has also yielded clinical benefits (25, 26). Regarding the anticancer mechanism, metformin appears to preferentially kill cancer-initiating/stem cells from glioblastoma (27), breast (28) and ovarian cancers (29) via AMP-activated protein kinase (AMPK) activation.

In contrast to the inhibitory action of metformin on tumor cells, here we demonstrate the direct effects of metformin on $CD8^+$ T cells, which eventually results in tumor growth inhibition. Metformin protects $CD8^+$ tumor-infiltrating lymphocytes (TILs) from apoptosis, and the multifunctionality of exhausted PD-1^-Tim-3^+CD8^+ TILs is restored via a shift from a central memory (TCM) to an effector memory T-cell (TEM) phenotype. This metformin-induced antitumor mechanism is therefore linked to marked changes in the characteristics of $CD8^+$ TILs within the tumor microenvironment.

Results

Metformin-Induced Tumor Rejection Depends on $CD8^+$T Cells. As metformin has been reported to decrease the rate of cancer incidence in type 2 diabetic patients, we at first examined whether

Significance

The multifunctional ability of CTLs is downregulated by interaction between immune-checkpoint molecules expressed on CTLs and their ligands expressed on cancer cells, referred to as immune exhaustion. The antibody-mediated, immune-checkpoint blockade turned out to a promising method for immunotherapy against advanced melanoma. Metformin, a drug prescribed for patients with type 2 diabetes, has been recognized to have anti-cancer effect. We found that $CD8^+$ tumor infiltrating lymphocytes (TILs) is a target of metformin. $CD8^+$ TILs inevitably undergo immune exhaustion, characterized by diminished production of multiple cytokines such as IL-2, TNFα, and IFNγ, followed by elimination with apoptosis. Metformin is able to counter the state. Along with conventional therapy, treatment of cancer patients with metformin may have a great advantage for cancer therapy.

Author contributions: H.U. designed research; S.E. and M.N. performed research; S.M., C.Y., E.N., and H.U. analyzed data; and H.U. wrote the paper.

The authors declare no conflict of interest.

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the drug could protect mice from methylchoranthrene-induced skin carcinogenesis. BALB/c mice were injected with 200 μg of methylchoranthrene on the right back and given 5 mg/mL metformin dissolved in the drinking water throughout the experiment. Significant inhibition of tumor development was observed in metformin-treated nondiabetic mice (Fig. S1*A*). We next attempted to determine whether metformin would be effective against an established solid tumor. Mice were intradermally injected with X-ray-induced RLmale1 leukemia cells and were provided oral metformin beginning on day 7. The tumors were gradually and completely rejected with no reappearance after metformin withdrawal. A rechallenge with more than twice the original number of the same tumor cells did not yield mass formation (Fig. 1*A*, *Left*), suggesting the generation of an immunologic memory response. Moreover, the antitumor effect was completely abrogated in SCID mice (Fig. 1*A*, *Right*), clearly demonstrating the necessity of T and/or B cells. Cytotoxic T lymphocytes (CTLs) specific for the tumor antigen peptide pRL1a (30) were generated in mice that rejected the tumor (Fig. S1*B*). Growth inhibition was observed with a metformin dose as low as 0.2 mg/mL (Fig. S1*C*). Of note, a previous report identified the achievement of plasma metformin concentrations of 0.45 and 1.7 μg/mL using 1 and 5 mg/mL of metformin, respectively, in drinking water (31); these plasma concentrations are similar to those in patients with diabetes treated using metformin (0.5–2 μg/mL). Administration of metformin beginning on day 0, the time point of tumor inoculation, resulted in more effective rejection than on day 7. Beginning treatment on day 10 and 13 was also effective, although the effect was less than on day 0 (Fig. S1*D*). Finally, as expected, $CD8^+$ but not $CD4^+$ T cells were proven to be responsible for the antitumor effect, because their depletion by mAb completely abrogated the response (Fig. 1*B*). Complete rejection by metformin was also observed with Renca (renal cell carcinoma), although partial but significant growth inhibition was observed with other tumors, 3LL (non small cell lung carcinoma), Colon 26 (intestinal carcinoma), and 4T1 (breast cancer) (Fig. S1 *E–H*).

Metformin Prevents Apoptosis of $CD8^+$TILs, Irrespective of Expression of PD-1 and Tim-3. Injection of a vaccine consisting of antigen (Ag) and adjuvant primes and generates specific T-cell immunity, mainly in draining lymph nodes near the injection site. However, we did not inject tumor antigens with any kind of adjuvant in Fig. 1. Therefore, it is possible that a unique process occurs at the tumor site and leads to antitumor immunity. Based on this notion, we focused on TILs throughout the experiment to clarify the associated mechanism. We found that total numbers of TILs dramatically increased when metformin administration was started on day 7, and that both $CD8^+$ and $CD4^+$ T cells were involved in the increment (Fig. 1 *C–E*). In particular, the number of $CD8^+$ TILs increased nearly fourfold. We considered the possibility that metformin may suppress expression of the immune exhaustion markers PD-1 and Tim-3 on $CD8^+$ TILs, thus avoiding immune exhaustion. Therefore, we investigated the expression of these markers on $CD8^+$ TILs derived from individual tumor-bearing mice (Fig. S1*B*). The number of $PD\text{-}1^-Tim\text{-}3^-CD8^+$ TILs decreased from day 7–10, irrespective of metformin use (Fig. S2*B*). The $PD\text{-}1^-Tim\text{-}3^+CD8^+$ TIL population increased progressively, whereas $PD\text{-}1^+Tim\text{-}3^-$ and $PD\text{-}1^+Tim\text{-}3^+CD8^+$ TILs remained stable. Metformin did not affect any subset populations (Fig. S2 *B–E*). However, we surprisingly found that a significant proportion of $CD8^+$TILs underwent apoptosis, detected by

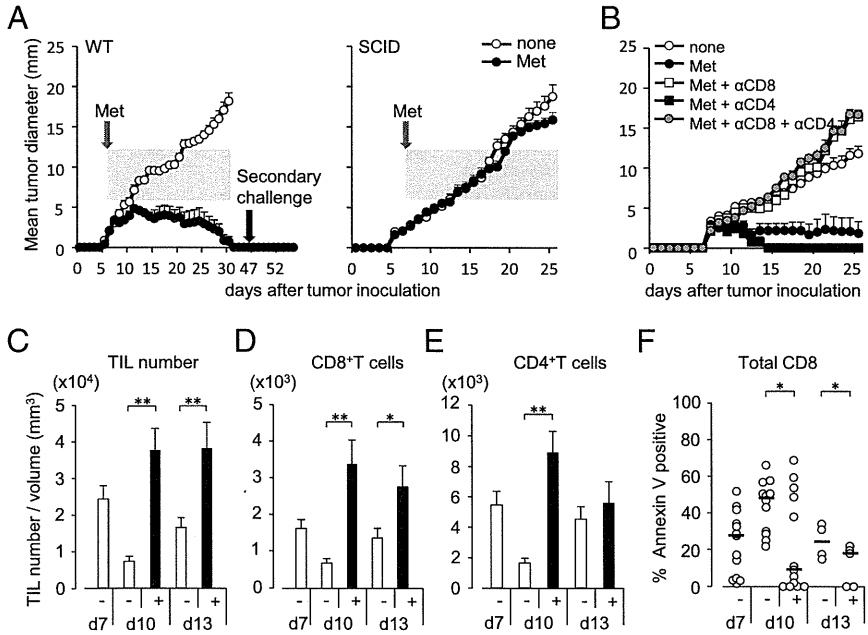

Fig. 1. Metformin suppressed tumor growth in vivo, depending on $CD8^+$ T cells. (*A*) On day 0, BALB/c WT or SCID mice were intradermally inoculated with 2×10^5 RLmale1 cells on the right back. The mice received 5 mg/mL metformin (Met) or not (none) dissolved in the drinking water. The duration of Met administration is indicated by the shaded rectangle. The mean diameter of each tumor was measured every day and the data are plotted with SE. On day 45, Met-treated WT mice, all of which had rejected the tumor, were rechallenged with 5×10^5 RLmale1 cells. $n = 6$ in each group. The results are representative of two independent experiments. (*B*) Mice inoculated with RLmale1 were treated with metformin (Met) or not (none), starting on day 7 and i.v. injected with anti-CD8 mAb and/or anti-CD4 mAb on the same day. Average tumor diameters are plotted with SE. $n = 5$ in each group. (*C–E*) Mice inoculated with RLmale1 cells were treated with Met (+) or not (–) from day 7. On day 7, 10 and 13, the tumor mass was isolated and TILs were recovered. The numbers of TILs per tumor volume (mm^3) were calculated. The numbers of TIL (*C*), $CD8^+$ (*D*), or $CD4^+$ (*E*) per tumor volume are depicted. Also, the populations of $CD8^+$TILs stained with Annexin V were plotted (*F*). All data were with SD ($n = 14$ on days 7 and 10, $n = 5$ on day 13). The horizontal bars indicate median values, and *P* values obtained by two-tailed Student's *t* test are shown as $*P < 0.05$, $**P < 0.01$ $n = 5$–14 in each group. Each symbol represents an individual mouse. The results depicted are a summary of three independent experiments.