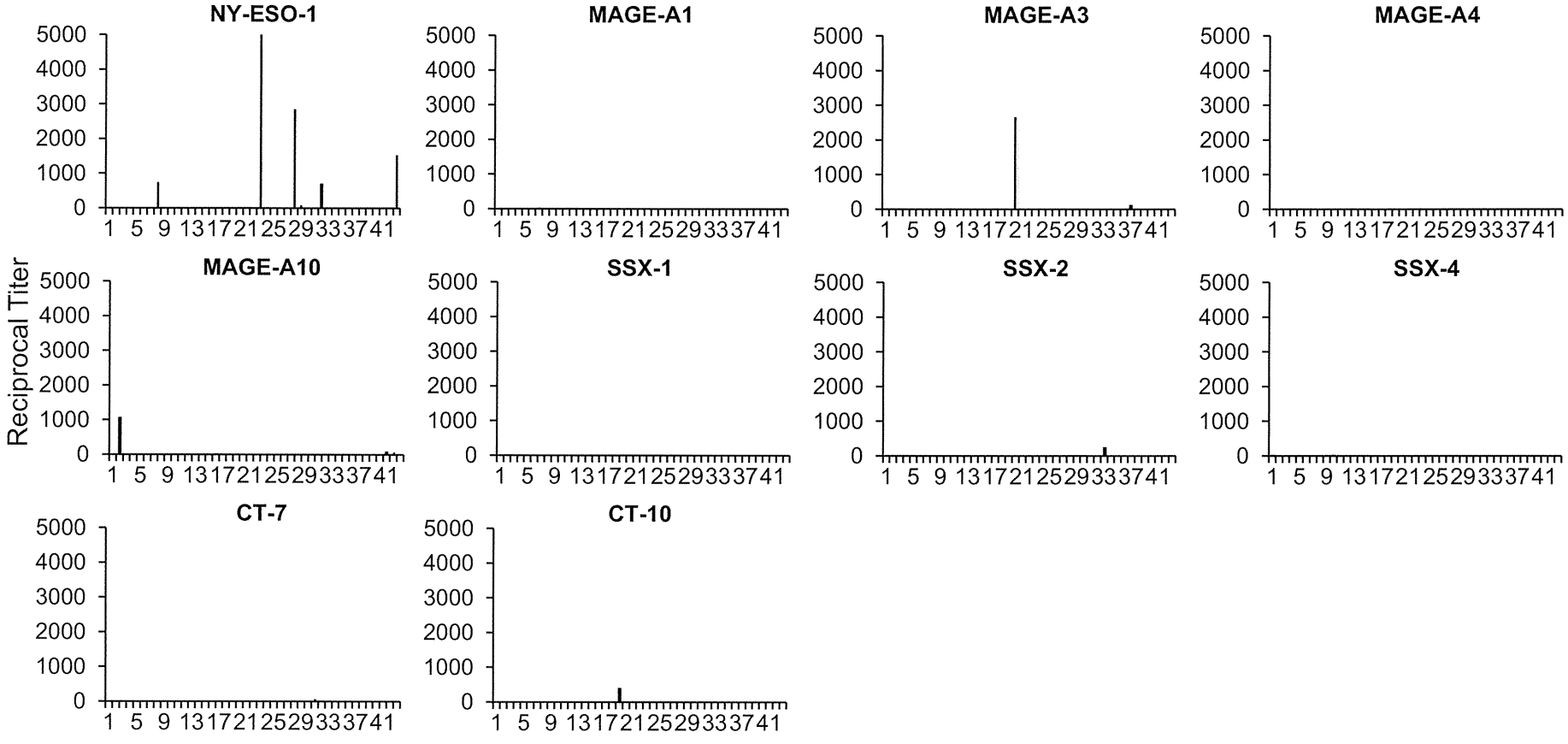
Figure 2


NY-ESO-1
MAGE-A1
MAGE-A3
MAGE-A4
MAGE-A10
SSX-1
SSX-2
SSX-4
CT-7
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Reciprocal Titer
5000
4000
3000
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1 5 9 13 17 21 25 29 33 37 41

Figure 3

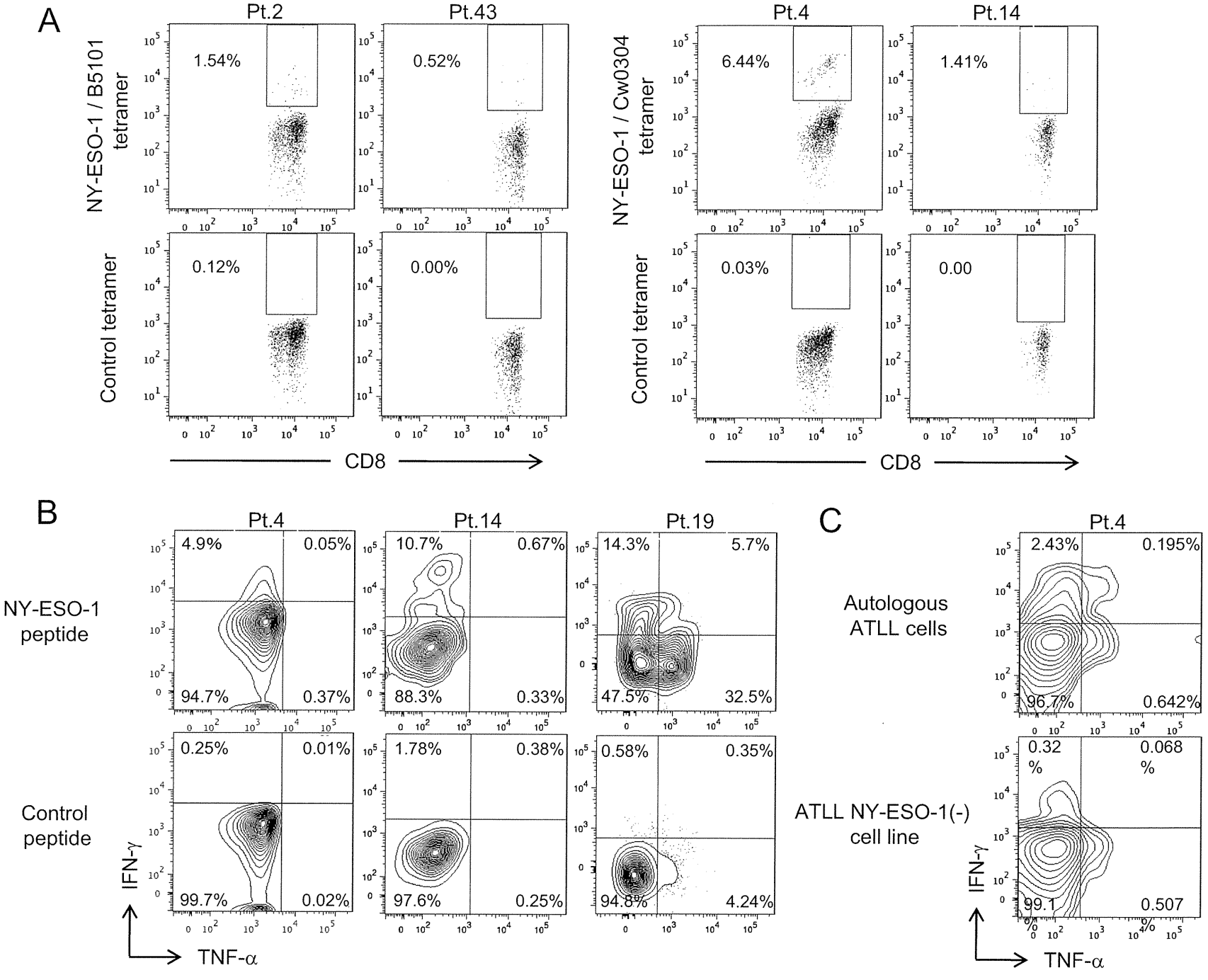
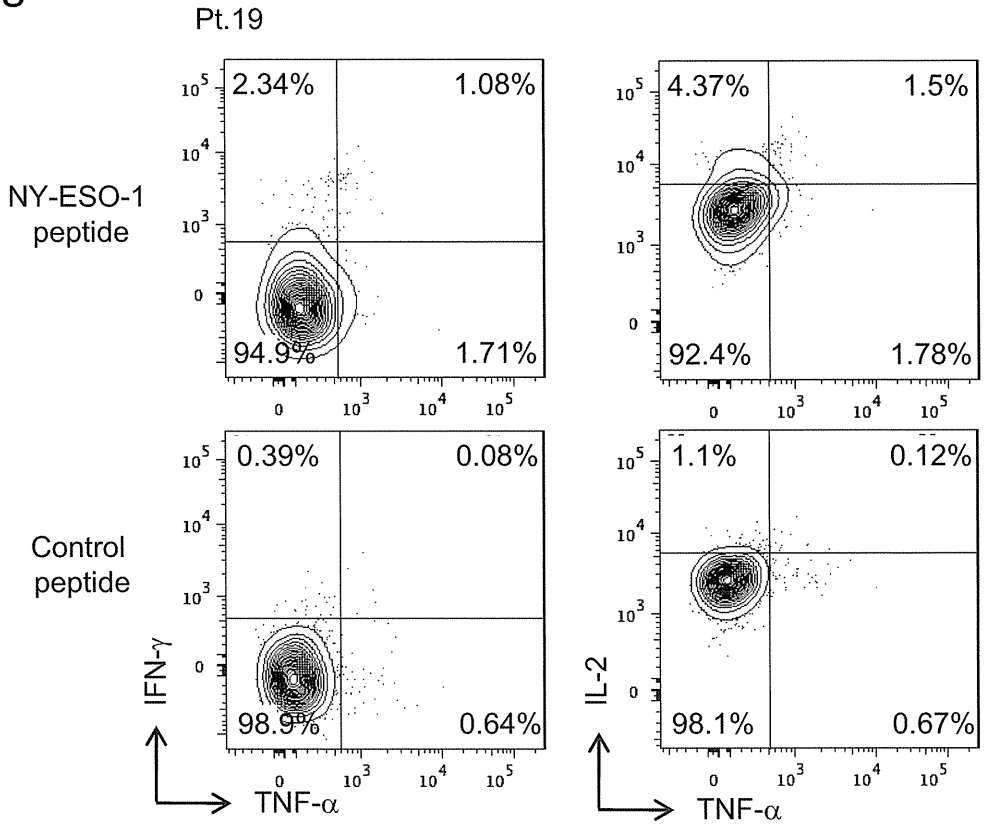

A
Pt.2
Pt.43
Pt.4
Pt.14
NY-ESO-1 / B5101 tetramer
NY-ESO-1 / Cw0304 tetramer
Control tetramer
1.54%
0.52%
6.44%
1.41%
0.12%
0.00%
0.03%
0.00
CD8
B
Pt.4
Pt.14
Pt.19
NY-ESO-1 peptide
Control peptide
4.9%
0.05%
94.7%
0.37%
10.7%
0.67%
88.3%
0.33%
14.3%
5.7%
47.5%
32.5%
0.25%
0.01%
99.7%
0.02%
1.78%
0.38%
97.6%
0.25%
0.58%
0.35%
94.8%
4.24%
IFN-γ
TNF-α
C
Pt.4
Autologous ATLL cells
ATLL NY-ESO-1(-) cell line
2.43%
0.195%
96.7%
0.642%
0.32 %
0.068 %
99.1
0.507
IFN-γ
TNF-α

Figure 4


Pt.19
NY-ESO-1 peptide
Control peptide
2.34%
1.08%
94.9%
1.71%
4.37%
1.5%
92.4%
1.78%
0.39%
0.08%
98.9%
0.64%
1.1%
0.12%
98.1%
0.67%
IFN-γ
TNF-α
IL-2
TNF-α

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Review Article

Immunopathogenesis of lymphoma: Focus on CCR4

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Evading immune surveillance is one of the common hallmarks of cancer. Herein we describe two major evasion mechanisms in lymphoma, focusing on regulatory T (Treg) cells and C-C chemokine receptor 4 (CCR4) expressed on these cells. First, the tumor cells themselves function as Treg cells, characterized by expression of CCR4, contributing to tumor survival by downregulating host immunity. Second, CCR4 ligands are produced by tumor cells, which attract other CCR4$^+$ Treg cells to the vicinity of the tumor. CCR4$^+$ adult T-cell leukemia/lymphoma is an example of the former phenomenon, and Hodgkin lymphoma of the latter, for which an almost identical immunopathogenesis has been reported in many types of cancer. Awareness of the importance of CCR4 allows the rational design of more effective cancer treatments. Accordingly, we have developed a defucosylated anti-CCR4 mAb, the first therapeutic agent targeting CCR4 to be used clinically for cancer. The therapeutic anti-CCR4 mAb represents a promising treatment method for patients with CCR4$^+$ neoplasms by directly killing the cancer cells, but could also be used as a novel treatment strategy for many types of CCR4$^-$ cancers to overcome the suppressive effect of CCR4$^+$ Treg cells. (*Cancer Sci* 2011; 102: 44–50)

The current World Health Organization (WHO) classification of lymphoid neoplasms is based on the use of all pertinent available information such as morphology, immunophenotype, genetic features, and clinical features, and a normal counterpart cell is postulated for each neoplasm.[1] B cell differentiation is relatively well understood, and B-cell neoplasms listed in the current WHO classification correspond well to stages of normal B cell differentiation. For instance, mantle cell lymphoma, follicular lymphoma, and marginal zone lymphoma correspond to pre-germinal center naïve B cells, germinal center B cells, and post-germinal center marginal zone B cells, respectively, and proliferate at their sites of origin, that is, mantle zone, germinal center, and marginal zone, respectively. In contrast, the classification of T-cell neoplasms, especially mature T-cell neoplasms, which are also called peripheral T-cell lymphomas (PTCL), remains challenging, with approximately 30–50% of cases considered "PTCL unclassifiable" (PTCL-not otherwise specified [NOS]), probably due to complex pathways of T-cell differentiation. Thus, the current PTCL classification may represent a provisional organization of syndromes rather than a list of distinct disease entities.[1]

Adult T-cell leukemia/lymphoma (ATLL)

Among PTCL, ATLL is a distinct disease entity caused by human T-cell leukemia virus type 1 (HTLV-1). Its diagnosis is confirmed by the monoclonal integration of HTLV-1 into neoplastic T-cells. HTLV-1 is transmitted mainly from mother to infant through breast milk.[2] The HTLV-1 receptor is the glucose transporter 1 (GLUT1) protein expressed ubiquitously.[3] Thus, HTLV-1 can infect different types of cells in addition to CD4$^+$ T-lymphocytes.[4,5] This fact gives rise to the simple question of why HTLV-1 causes malignant transformation only of CD4$^+$ T-lymphocytes, especially CD4$^+$CD25$^+$C-C chemokine receptor 4 (CCR4)$^+$ cells.[4–7] CCR4 is known to be expressed selectively on regulatory T (Treg) and type 2 helper T (Th) cells.[8–13]

ATLL and Treg cells

CD4$^+$ Th lymphocytes represent a heterogeneous population of cells that play an essential role in adaptive immunity. These cells include effector subsets, such as Th1, Th2, Th17, and follicular helper T (Tfh) cells, which protect against pathogens, and Treg cells, which protect against effector responses to autoantigens and also against over-exuberant responses to exogenous antigens when they may become dangerous for the host. Each effector subset develops through the actions of different transcription factors, such as T-bet for Th1,[14] GATA-3 for Th2,[15] RORC for Th17,[16] and bcl-6 for Tfh,[17,18] with FOXP3 being the key transcription factor for the development and function of Treg cells.[9,19–21] Of the several types of Treg cells, both naturally occurring and induced Treg cells are well characterized; the former are generated in the thymus and the latter from naive T cells in the periphery (Fig. 1).[22,23] Regarding surface phenotypes, Treg cells express CD25 and CCR4, in addition to CD4.[8–12] Because tumor cells from most ATLL patients also have this CD4$^+$CD25$^+$CCR4$^+$FOXP3$^+$ phenotype,[6,7,24,25] based on their phenotypic characteristics, these tumors may originate from CD4$^+$CD25$^+$CCR4$^+$FOXP3$^+$ Treg cells. In addition, we have shown that CCR4$^+$ ATLL cells from a subset of patients do indeed function as Treg cells in an autologous setting.[26] This finding provides some insight into why HTLV-1 causes only CD4$^+$CD25$^+$CCR4$^+$ lymphocytes to develop into neoplasia.

Why does HTLV-1 cause malignant transformation of only CD4$^+$CD25$^+$CCR4$^+$ lymphocytes?

As mentioned above, HTLV-1 infects different types of cells, including Treg cells, through GLUT1 in a cell to cell manner. *Tax* and other viral genes such as *HBZ* mediate growth promotion in HTLV-1-infected cells, which express viral-associated antigens derived from core, envelope, polymerase, or Tax on their surface. This implies that infected cells must face the host antiviral immune responses.[27] Therefore, it is expected that HTLV-1 infected cells should be recognized and eradicated by HTLV-1-specific CTL. Thus, HTLV-1 infected Treg cells may have a survival advantage compared to other types of cells, by

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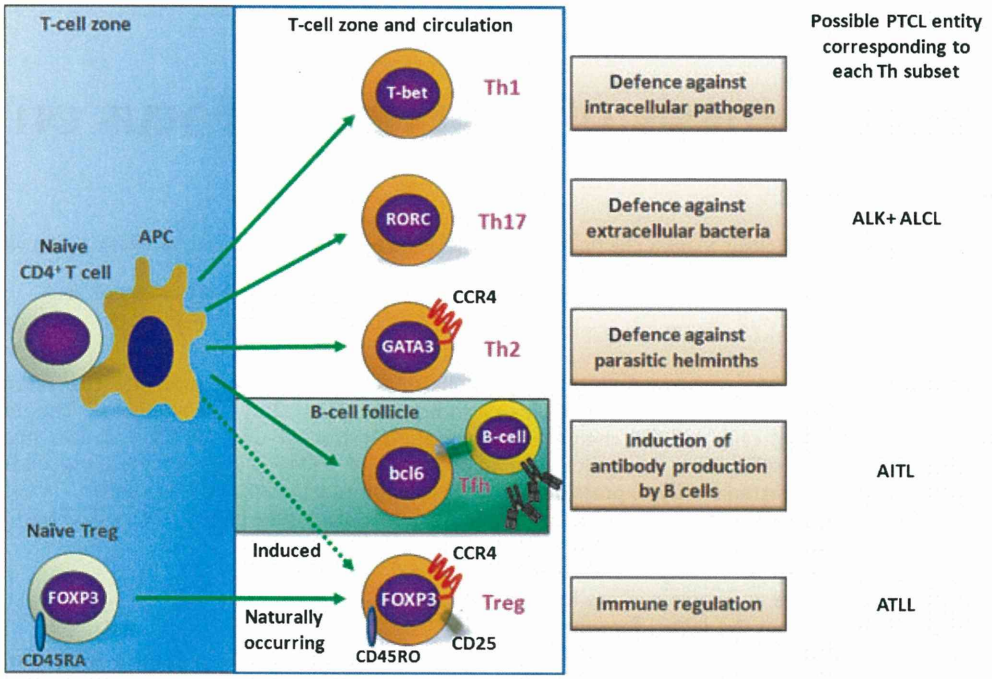

Fig. 1. $CD4^+$ T helper (Th) lymphocyte differentiation and corresponding peripheral T-cell lymphomas (PTCL). Different types of cytokines produced by dendritic cells and other sources facilitate the differentiation of naive $CD4^+$ T cells into Th lymphocyte subsets such as Th1, Th2, Th17, follicular helper T (Tfh), and regulatory T (Treg) cells. Each Th subset differentiates through the actions of different specific transcription factors, and plays an essential role in adaptive immunity. Possible PTCL disease entities corresponding to each Th subset are indicated. AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; APC, antigen presenting cells.

suppressing host immune responses against themselves. As a result, HTLV-1 infected $CD4^+CD25^+CCR4^+$ Treg cells should preferentially survive, and gradually increase in number. Furthermore, the accumulation of additional crucial genomic and/or epigenomic alterations could cause HTLV-1 infected cells to change into clonally proliferating ATLL cells. Collectively, the natural progression from HTLV-1 infection to ATLL development is illustrated in Figure 2, based on that proposed by Matsuoka *et al.*[4,5] Silencing HTLV-1 associated antigens, such as Tax, is a generally accepted mechanism to avoid immunity,[27] although it fails to explain selective tumorigenesis caused by HTLV-1 that occurs in $CD4^+CD25^+CCR4^+$ lymphocytes.

PTCL-NOS

These lymphomas make up a heterogeneous category of mature T-cell lymphomas, which do not correspond to any of the specific defined entities of mature T-cell lymphoma in the current WHO classification.[1] Approximately 30–40% cases of PTCL-NOS are $CCR4^+$,[28–30] and CCR4 expression is an independent and significant unfavorable prognostic factor in these patients.[29] Ohshima *et al.*[28] also reported that CCR4 expression was an unfavorable prognostic factor for this type of lymphoma. Together with the fact that most ATLL, which belong to the group with the most unfavorable prognosis among PTCL,[31] are positive for CCR4, these findings raise the question why CCR4 expression is an unfavorable prognostic factor for PTCL-NOS. Based on genomic profiling, Nakagawa *et al.*[30] proposed that PTCL-NOS could be divided into two groups, one with and one without genomic alterations, the former being significantly associated with shorter overall survival time, and frequently showing expression of CCR4 (16 of 26 [61.5%] *versus* ONE of 22 [4.5%], respectively; $P < 0.0001$). This study provided new insights into our understanding of CCR4-expressing PTCL, and possible answers to the above question.

Why is CCR4 expression an unfavorable prognostic factor for PTCL-NOS?

Although PTCL-NOS are particularly heterogeneous, the $CCR4^+$ subset may be a distinct disease entity originating from a Treg cell. The finding that $CCR4^+$ PTCL-NOS had significantly higher expression of CD25 compared to $CCR4^-$ PTCL-NOS (nine of 13 [69.2%] *versus* 6 of 31 [19.4%], respectively; $P = 0.0038$)[29] is consistent with a possible association of CCR4-expressing PTCL with Treg cells. It has been generally accepted that development of cancer, including PTCL, is a multistep process requiring the accumulation of multiple genetic and epigenetic alterations.[32,33] Typically, a T cell acquiring certain initial genetic/epigenetic alterations expresses tumor antigens derived from the resulting mutated genes or modified proteins, and thus would face host immune responses.[34] Probably, only PTCL cells that originate from a Treg cell have adequate time to accumulate the full bevy of genetic/epigenetic alterations required for malignant transformation, because compared to other types of T cells, they are immunologically privileged by their ability to downregulate antitumor immune responses. As a result, PTCL originating from a $CCR4^+$ Treg cell may tend to be ''PTCL-NOS with genomic alterations'', and have significantly worse prognosis. In contrast, the PTCL that originate from non-Treg cells may tend to be ''PTCL-NOS without genomic alterations''. Notably, this hypothetical scenario of ''PTCL-NOS with genomic alterations'' is extremely similar to that for ATLL, in which most cases are $CCR4^+$ (Fig. 2).[7] In fact, Nakagawa *et al.* reported that not only clinicopathological features, including overall survival curves, but also the genomic profiles of ''PTCL-NOS with genomic alterations'', were very similar to lymphoma-type ATLL.[30,35] This finding supports the hypothesis that ''PTCL-NOS with genomic alterations'' and ATLL have an identical immunopathogenesis, that is, they both originate from a $CCR4^+$ Treg cells. In

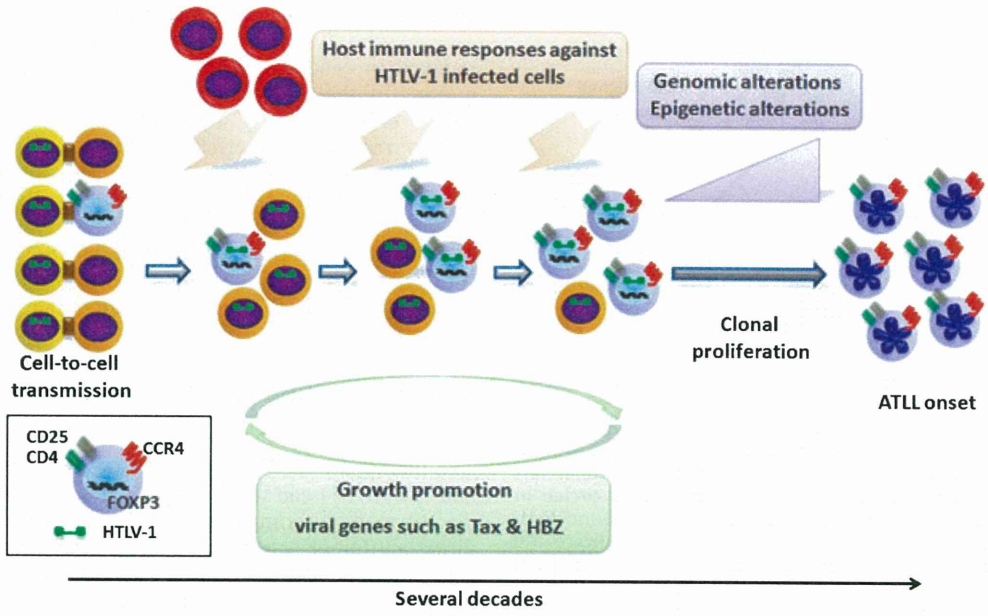

Fig. 2. Natural progression from HTLV-1 infection to adult T-cell leukemia/lymphoma (ATLL) development. HTLV-1 infects various types of cells including regulatory T (Treg) cells through GLUT1 in a cell-to-cell manner, and viral-associated genes promote growth of the infected cells. HTLV-1 infected cells express viral-associated antigens, and are eradicated by HTLV-1-specific CTL, whereas only HTLV-1 infected Treg cells should have an immunological survival advantage compared to other types of HTLV-1 infected cells, due to their suppression of host immune responses. As a result, HTLV-1 infected Treg cells should preferentially survive, and gradually increase in number. Furthermore, the accumulation of additional critical genomic and/or epigenomic alterations will cause development of the HTLV-1 infected cell into clonally proliferating ATLL cells. After a prolonged asymptomatic period of 50–70 years, ATLL development occurs in approximately 5% of infected individuals, but most HTLV-1 infected individuals remain life-long asymptomatic carriers.

addition, the significant correlation between the expression levels of *CCR4* and *FOXP3* mRNA in affected lymph node cells obtained from patients with PTCL-NOS[29] is consistent with a possible association of $CCR4^+$ PTCL-NOS with Treg cells.

Angioimmunoblastic T-cell lymphoma (AITL) and anaplastic lymphoma kinase $(ALK)^+$ anaplastic large cell lymphoma (ALCL)

Recent studies have provided evidence that AITL is a neoplasm that originates from Tfh cells. These cells home to B-cell follicles and induce antibody production by B cells. They express chemokine receptor CXCR5, which allows their migration into the CXCL13-rich B-cell follicles of secondary lymphoid tissues. Expression of CXCR5 as well as BCL6 and CD10 is observed in the majority of AITL cases.[36,37] In addition, the AITL gene signature has been shown to be enriched in genes of normal Tfh cells.[38,39] The cellular origin of AITL from Tfh cells provides a rational model to explain several of the peculiar pathological and biological features inherent to this disease, that is, the intimate association with germinal centers in early disease stages, and the polyclonal hypergammaglobulinemia. Follicular helper T cells are unique regulatory cells that suppress the activation of conventional $CD4^+$ T cells, particularly Th1 cells,[40] a finding that could at least partly explain the immune dysfunctions observed in AITL patients (Fig. 1).

Although the expression of Th17-associated molecules in ALK^+ ALCL was noted in the gene expression profiling carried out by Iqbal *et al.*,[41] it may not fully explain several of the peculiar pathological and biological features of ALK^+ ALCL,[1] other than its expression of the cytotoxic molecules (Fig. 1).

Hodgkin lymphoma (HL)

Hodgkin lymphoma is characterized by the presence of a small number of tumor cells in a rich background of T and B cells, macrophages, and other inflammatory cells.[1] The question why a very small number of HL cells can survive in the face of a large excess of host immune cells has remained open for a long time. HL cells express high levels of the CCR4 ligand thymus and activation-regulated chemokine (TARC)/CCL17,[42] and an elevated serum level of TARC/CCL17 was reported as an unfavorable prognostic factor in patients with HL.[43] Taking these findings into consideration, we have shown that HL tumor cells attracted $CD4^+CCR4^+$ T cells by interaction with the CCR4 ligands they produce, namely, TARC/CCL17 and macrophage-derived chemokine (MDC)/CCL22. The migratory $CD4^+$ cells attracted by HL tumor cells were hyporesponsive to T-cell receptor stimulation and suppressed the activation and proliferation of effector $CD4^+$ T cells in an autologous setting *in vitro*. Furthermore, double staining showed that HL cells in the affected lymph nodes were surrounded by a large number of lymphocytes expressing both CCR4 and FOXP3.[11] Collectively, these findings imply that the migratory cells induced by HL function as Treg cells to create a favorable environment for the tumor cells to escape from host immunity (Fig. 3).

Epstein–Barr virus (EBV)-associated lymphoproliferative disorders

Epstein–Barr virus has been shown to contribute to the development of several types of mature B, T, and natural killer (NK) cell lymphomas. Among B-cell lymphomas, EBV is involved in the pathogenesis of lymphomas such as endemic Burkitt lymphoma, many types of immunodeficiency-associated lymphoproliferative disorders, plasmablastic lymphoma, and EBV^+ diffuse large B-cell lymphoma (DLBCL) of the elderly.[1] We focus on the last of these here. Nakayama *et al.*[44] reported that EBV-infected B cells acquire the ability to produce TARC/CCL17 and MDC/CCL22 through latent membrane protein 1 (LMP1)-mediated activation of nuclear factor κB, and suggested that the production of these factors, which attract $CCR4^+$ Treg cells,

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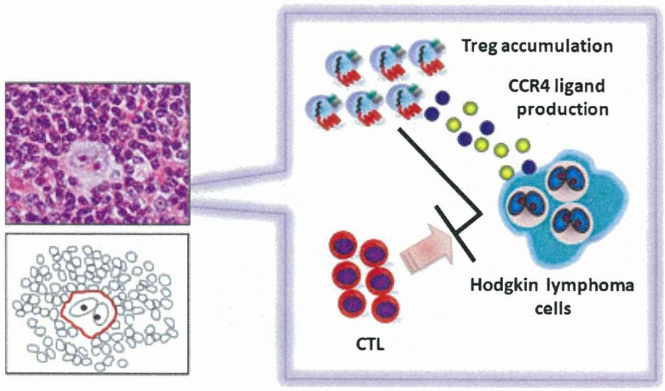

Fig. 3. Specific recruitment of CCR4$^+$ regulatory T (Treg) cells in Hodgkin lymphoma (HL) fosters immune privilege. Hematoxylin–eosin staining of affected lymph node from a patient with HL (upper left panel). A HL tumor cell termed a "Reed–Sternberg cell" is circled in red; all other cells with nuclei outlined in black are background non-tumor cells (lower left panel). Thus, the two left panels indicate the histological characteristics of HL, that is, the presence of a small number of tumor cells in a rich background of T and B cells, macrophages, and other inflammatory cells. HL tumor cells produce TARC/CCL17 and MDC/CCL22, and migratory CCR4$^+$ cells induced by HL cells function as Tregs, so that these cells prevent the host immune system from attacking tumor cells, thereby create a favorable environment for the latter (right panel).

may help EBV-infected B cells to evade immune surveillance by the host. In the context of this scenario, Takegawa *et al.* reported that LMP1-expressing tumor cells were typically large in size and selectively positive for TARC/CCL17 and MDC/CCL22 in EBV$^+$ DLBCL of the elderly. Moreover, both CCR4$^+$ and FOXP3$^+$ cells were abundant among the infiltrating T cells of EBV$^+$ DLBCL of the elderly.[45] These findings indicate that the migratory cells induced by LMP1-expressing large-sized tumor cells probably function as Treg cells to create a favorable environment for the tumor cells to escape from host immunity, very similar to the situation with HL (Fig. 3). In fact, a frequent histological feature in EBV$^+$ DLBCL is an abundant reactive cellular infiltration into the tumor,[46] and this feature is also quite reminiscent of HL.

CCR4$^+$ Treg cells in cancer other than lymphoma

It has been reported that Treg cells accumulate in tumor tissues via interaction of their CCR4 and its ligands produced by cells of the tumor microenvironment. It was proposed that Treg-mediated immunosuppression is a crucial tumor immune-evasion mechanism in many types of cancer such as ovarian, gastric, breast, and pancreatic, and may be one of the main obstacles to successful tumor immunotherapy.[10,47,48] That is to say, the immunopathogenesis of many types of cancer seems to be almost identical to HL (Fig. 3).

CCR4 as a novel molecular target for immunotherapy of cancer

Recognition of the importance of CCR4 on tumor and Treg cells in different types of cancer and in the microenvironment allows the rational design of more effective treatments. Accordingly, we have proposed CCR4 as a novel molecular target of cancer immunotherapy, and developed therapeutic chimeric (KM2760) and humanized (KW-0761) anti-CCR4 mAbs.[49,50] Antibody-dependent cellular cytotoxicity (ADCC) is one of the most important mechanisms of action of therapeutic mAb;[51–53] however, ADCC depends on the cytotoxic activity of effector cells, such as NK cells and monocytes/macrophages, which are commonly qualitatively suppressed and quantitatively reduced in cancer patients. To overcome this problem, the Fc regions of the therapeutic anti-CCR4 mAbs were defucosylated, to enhance ADCC by increasing antibody binding affinity to Fcγ receptors on effector cells.[12,49,54] We have reported that robust ADCC of the therapeutic defucosylated anti-CCR4 mAb mediated by autologous effector cells is indeed triggered in some ATLL, PTCL-NOS, and advanced/refractory cutaneous T-cell lymphoma patients *in vitro*.[25,55] This mAb also showed significant antitumor activity in disseminated and non-disseminated CCR4-positive lymphoma models in SCID mice.[55–57]

Humanized mouse model to evaluate human immunotherapy

The therapeutic defucosylated anti-CCR4 mAbs (both KM2760 and KW-0761) can induce highly enhanced ADCC activity, but do not mediate complement-dependent cytotoxicity or possess direct antitumor activities.[49,50] Because there were no suitable small animal models to evaluate human ADCC *in vivo*, due to species incompatibilities, we established a "humanized mouse," in which human immune cells from healthy individuals function as ADCC effector cells against allogeneic tumor cell lines, using NOD/Shi-*scid*, IL-2Rγ^{null} (NOG) mice[58,59] as recipients. In this model, the therapeutic anti-CCR4 mAb showed potent antitumor activity by human ADCC.[60] Using this humanized mouse model, we had the opportunity to undertake more appropriate preclinical evaluation of many types of mAb-based immunotherapy, although in the initial study, we could not completely exclude non-specific allogeneic immune responses because target and effector cells were obtained from different individuals. To overcome the subsequent problems, we have established a primary human tumor-bearing NOG mouse model, in which autologous human immune cells are engrafted and mediate ADCC but in which endogenous murine cells are unable to mediate ADCC. In that study, we used primary ATLL cells bearing NOG mice.[61] Figure 4 includes images of both an untreated NOG mouse with primary ATLL cells and a mouse that received the therapeutic anti-CCR4 mAb after inoculation with ATLL cells. The therapeutic anti-CCR4 mAb showed significant antitumor activity against primary ATLL cells by robust ADCC mediated by autologous effector cells from the same patients in NOG mice *in vivo*.[61] The study was the first to report a mouse model in which a potent antitumor effect of the therapeutic mAb against primary tumor cells is mediated by autologous human immune cells. This approach should make it possible to model the human immune system active in mAb-based immunotherapy *in vivo*, and thus to carry out more appropriate preclinical evaluations of novel therapeutic mAbs.

Clinical development of KW-0761

Based on the promising results of this preclinical work, and as an outcome of the success of this translational research, we have already completed a phase I clinical trial of KW-0761, in a single-agent, dose-escalation, multicenter study for patients with relapsed CCR4$^+$ T-cell leukemia/lymphoma in Japan (http://www.clinicaltrials.gov, Identifier: NCT00355472).[62] Importantly, this phase I study was the first clinical trial to examine the safety and efficacy of a next-generation defucosylated therapeutic antibody against cancer. Although the number of patients in this trial was small, it is noteworthy that objective responses were achieved in 31% of patients, with 13% complete responses. This is a particularly promising result as the response rate of relapsed patients with ATLL to conventional chemotherapy with

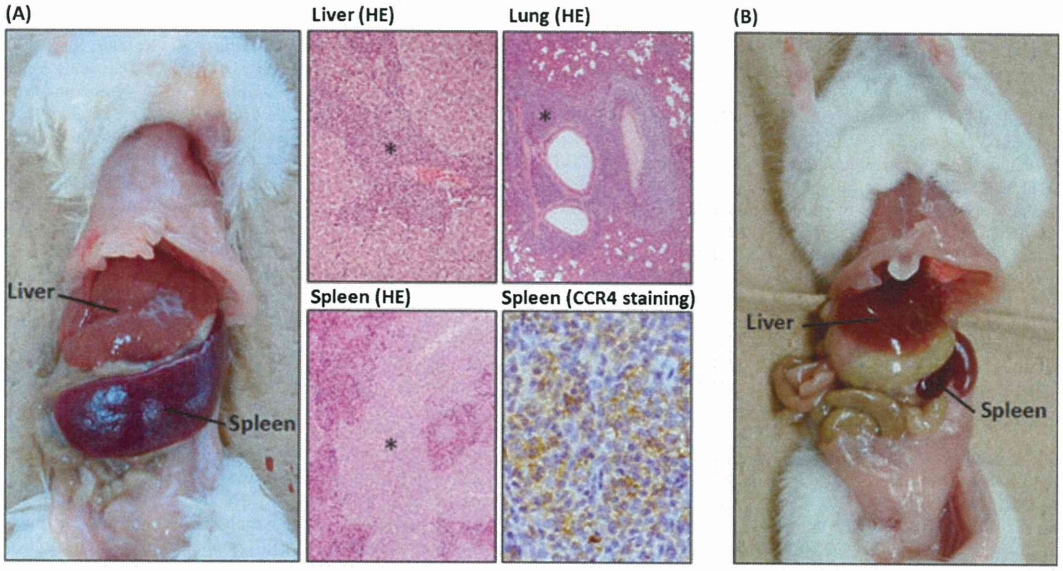

Fig. 4. Macroscopic observations and histological findings of a primary adult T-cell leukemia/lymphoma (ATLL) cell-bearing NOD/Shi-*scid*, IL-2Rγ^{null} (NOG) mouse. (A) Primary ATLL cell-bearing NOG mouse. Both the liver and spleen of a NOG mouse are enlarged. Immumohistological analysis revealed that the liver, lung, and spleen were infiltrated by CCR4$^+$ ATLL cells and the normal architecture of each organ was destroyed. Asterisks indicate tumor infiltrating region. (B) In contrast, primary ATLL cell-bearing NOG mouse treated with the therapeutic anti-CCR4 mAb has no morbid lesions detectable macroscopically.

a single agent is reportedly extremely low.[63] Clinical responses were observed even at 0.01 mg/kg, which is approximately 1/1000 of the rituximab dose.[64,65] The clinical effect observed at the 0.01 mg/kg dose of KW-0761 would be consistent with the concept of using defucosylation of therapeutic mAb to enhance ADCC. The phase I trial determined the recommended phase II dose to be 1.0 mg/kg, which is 1/10 of optimal rituximab dose of 10 mg/kg (i.e. 375 mg/m^2). Therefore, we subsequently carried out both a phase II clinical trial of KW-0761 against relapsed ATLL (http://www.clinicaltrials.gov, Identifier: NCT00920790), and a multicenter, randomized, open-label, parallel group study to compare mLSG15[66] combined with KW-0761 to mLSG15 alone in subjects with untreated ATLL (http://www.clinicaltrials.gov, Identifier: NCT01173887). In the USA, a phase I/II clinical trial of KW-0761 against PTCL has also been carried out (http://www.clinicaltrials.gov, Identifier: NCT00888927).

Conclusions

Cancer is a complex collection of distinct genetic diseases sharing hallmarks such as self-sufficiency in growth signaling, evading apoptosis, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis, evading immune surveillance, and stress phenotype.[32,33] Of those hallmarks, we focused on ''evading immune surveillance'', and here we propose two major evasion mechanisms in lymphoma. First, tumor cells themselves function as Treg cells, contributing to tumor survival in the face of the host immune response; second, ligands for CCR4 are produced by tumor cells and/or the tumor microenvironment, and then attract CCR4-expressing Treg cells to the tumor, where they create a favorable environment for tumor cells to survive despite host immune recognition. CCR4$^+$ ATLL is representative of the former, and HL of the latter, with respect to which, it has been generally accepted that increased Treg cells in the tumor microenvironment play an important role in tumor escape from host immunity in several different types of cancer. Moreover, Treg cells infiltrating the tumor may represent one of the main obstacles to successful tumor immunotherapy. Therefore, depletion of Treg cells in the vicinity of tumors is a potentially promising strategy for boosting tumor-associated antigen-specific immunity.[34,47,48] We showed that our therapeutic anti-CCR4 mAb actually did deplete Treg cells *in vitro*,[11,25] and furthermore, also had this activity *in vivo* in humanized mice.[60] Collectively, these data suggest that therapeutic defucosylated anti-CCR4 mAb may soon become a promising treatment for patients with CCR4$^+$ neoplasms by directly killing the tumor cells. Moreover, in the near future, they could also be used as a novel strategy for treatment of many other types of cancers to overcome the suppressive effect of CCR4$^+$ Treg cells on the host's immune response to tumor cells.

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Disclosure Statement

The authors have no conflict of interest.

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Antibody therapy for Adult T-cell leukemia–lymphoma

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Abstract Adult T-cell leukemia–lymphoma (ATL) has a very poor prognosis. Since there currently are limited treatment options for ATL patients, several novel agents are being developed and tested clinically. Antibody therapy against ATL was initially started with interleukin-2 receptor α-subunit, CD25, as a target molecule in the late 1980s, and is currently ongoing. CC chemokine receptor 4 (CCR4) was postulated as a novel molecular target in ATL antibody therapy, and humanized anti-CCR4 mAb (KW-0761), whose Fc region was defucosylated to enhance antibody-dependent cellular cytotoxicity, was developed. A phase I study of KW-0761 in relapsed ATL and peripheral T-cell lymphoma was started in 2006, and a subsequent phase II study was completed in 2010. KW-0761 showed a clinically meaningful antitumor activity in patients with relapsed ATL, with an acceptable toxicity profile. The prognosis of ATL patients should be improved in the near future with clinical applications of novel treatment strategies, including those involving KW-0761 and other promising antibody therapies targeting CD25 or CD30.

Keywords CCR4 · CD25 · ADCC · KW-0761

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1 Introduction

Adult T-cell leukemia–lymphoma (ATL) is a peripheral T-cell neoplasm caused by human T-cell leukemia virus type-1 (HTLV-1) [1–3] that has a very poor prognosis. The clinical features of 818 ATL patients with a mean age of 57 years, newly diagnosed from 1983 to 1987, were analyzed by Shimoyama [4] who demonstrated that the median overall survival (OS) was 6.2 and 10.2 months for acute and lymphoma type ATL, respectively.

2 Conventional chemotherapy for ATL

A most recent phase III trial for previously untreated aggressive ATL (acute, lymphoma, or unfavorable chronic type), with enrollment between 1998 and 2003, demonstrated that dose-intensified multidrug chemotherapy, including vincristine, cyclophosphamide, doxorubicin, prednisone, ranimustine, vindesine, etoposide, and carboplatin (VCAP-AMP-VECP), was superior to CHOP-14 and resulted in a median OS of 12.7 months, and an OS at 3 years of 24% [5]. Comparing two reports of Shimoyama [4] and Tsukasaki et al. [5], chemotherapy for ATL seems to have been improved. However, some of the ATL patients who were in good physical condition, relatively young and with preserved organ functions, could be enrolled into the later VCAP-AMP-VECP study, which likely might lead to a prolonged OS. A vast knowledge in molecular biology and oncogenesis of ATL has been accumulated since its recognition as a distinct disease in 1977 [1], and discovery of its causative virus in 1980–1982 [2, 3, 6], however, this knowledge does not seem to have contributed extensively to an improved clinical prognosis of ATL patients. Conventional chemotherapy seems to have reached its attainable limits of efficacy in ATL.

3 Antiviral therapy for ATL

Antiviral therapy, consisting of a combination of zidovudine (AZT) and interferon (IFN), was initially reported with a median OS of 3.0 months in 19 patients with acute or lymphoma type ATL [7]. Recently, Bazarbachi et al. proposed—based on a meta-analysis—that AZT/IFN should be considered the gold standard of first-line therapy for patients with acute, chronic, or smoldering types of ATL. They reported a median OS of 9 months for acute type ATL treated with AZT/IFN [8]. Considering this report together with the results of conventional chemotherapy, such as VCAP-AMP-VECP [5], it is difficult to conclude that AZT/IFN therapy should be considered as a standard first-line therapy in acute ATL [9]. As for chronic and smoldering types of ATL, Bazarbachi et al. reported 100% OS beyond 5 years, even though the number of patients in their study ($n = 17$) was small. This OS is surprisingly good, considering the report from Takasaki et al. that a 5-year OS was 47.2% in patients with chronic and smoldering types of ATL who were mainly observed by a watchful waiting policy until disease progression ($n = 90$) [10]. Considering the promising but preliminary nature of the results by Bazarbachi et al., the Japan Clinical Oncology Group is now planning a randomized phase III study that will compare the outcome of AZT/IFN versus watchful waiting in patients with favorable chronic and smoldering types of ATL. This study will establish the standard care for patients with non-aggressive ATL. On the other hand, it appears that antiviral therapy alone is likely to have the same limited efficacy in aggressive ATL as does conventional chemotherapy.

4 Allogeneic hematopoietic stem cell transplantation in ATL

Allogeneic hematopoietic stem cell transplantation in ATL has evolved over the last decade into a potential approach to bring complete cure to ATL patients [11–13]. Recently, Hishizawa et al. [14] reported a Japanese nationwide retrospective study of allogeneic stem cell transplantation in ATL, in which the unadjusted 3-year probability of OS was 33% (95% CI 28–38%), which seems promising. The study revealed that an age higher than 50 years was significantly associated with lower survival rates by a multivariate analysis, even though ATL has a long latency and occurs in elderly individuals with a median age of 60 years. The study also revealed that a status other than complete remission (CR) was significantly associated with lower survival rates by multivariate analysis, even though the CR rates of patients treated with VCAP-AMP-VECP and CHOP-14 were only 40% (95% CI 27.6–54.2%) and 25% (95% CI 14.5–37.3%), respectively [5]. These results indicate that only a small fraction of ATL patients currently benefit from allogeneic stem cell transplantation. Collectively, the development of alternative treatment strategies for ATL patients is an urgent issue.

5 Development of novel agents in the treatment of ATL

There are currently limited treatment options for ATL patients, as mentioned above, although several novel agents are currently being developed and tested clinically. Bortezomib, which inhibits the proteasome, is now widely used for relapsed or refractory multiple myeloma [15]. This agent induces cell death in HTLV-1-associated cell lines and in primary ATL cells in vitro via multiple pathways, including NF-kB inhibition [16, 17]. A phase II study of bortezomib in relapsed/refractory ATL is currently ongoing (UMIN000004061). The immunomodulatory agent lenalidomide is active in a variety of hematological malignancies, including multiple myeloma [15] and B-cell lymphomas. A dose-escalation study to assess the safety of lenalidomide in patients with advanced ATL and peripheral T-cell lymphoma (PTCL) is also currently ongoing (ClinicalTrials.gov: NCT01169298). In addition, other agents, including the histone deacetylase inhibitor vorinostat and the folate analog pralatrexate, are now being considered for clinical trials in ATL patients, whereas the present paper is focused on the development of antibody therapy for ATL.

6 Antibody therapy for hematological malignancies

The use of therapeutic monoclonal antibodies (mAb) in the treatment of cancer has evolved into a promising approach over the last decade. In the clinical field of hematological malignancies, development of the chimeric anti-CD20 mAb rituximab has changed the standard therapy in patients with B-cell lymphomas and has markedly improved their prognoses [18, 19]. The success of anti-CD20 therapy in B-cell lymphoma is prompting investigators to search for a similarly efficacious mAb in T-cell lymphoma.

7 CD25 targeting therapy in ATL

7.1 Unmodified anti-CD25 antibody therapy

Antibody therapy against ATL was initially started by Waldmann et al. Because ATL cells constitutively express interleukin-2 (IL-2) receptor α-subunit, CD25 [20, 21], they assigned this molecule as a target of antibody therapy.

They developed a mouse IgG2a mAb, termed anti-Tac, that recognized CD25 [22]. Since this antibody blocks the binding of IL-2 to its receptor on ATL cells, they postulated that anti-Tac mAb prevented ATL cell proliferation. Among 9 ATL patients treated with anti-Tac mAb, objective responses were observed in two patients (22%), including one CR [22]. Further evaluation of this agent in 19 ATL patients was performed, and objective responses were observed in six patients (32%), including 2 CRs and 4 partial remissions (PRs) [23]. Although the use of unmodified anti-Tac mAb in the treatment of ATL seems encouraging, there are several problems. One obstacle to the success of this antibody was provided by the observation that the ATL cells of most patients in the aggressive phase no longer produce IL-2 or require IL-2 for their proliferation and survival [24]. Another obstacle was the appearance of antibodies against anti-Tac mAb, i.e. human anti-mouse antibody (HAMA). HAMA was observed in 11.1% (1/9) of patients in the former [22] and in 15.8% (3/19) of patients in the latter study [23]. The appearance of HAMA is a common obstacle to success of therapeutic mouse mAb such as anti-Tac [25]. Rodent mAbs have short half-lives in humans and induce an immune response that neutralizes their therapeutic effect. Furthermore, the murine antibodies only weakly recruit human effector cells or elements, leading to insufficient ADCC (antibody-dependent cellular cytotoxicity) or CDC (complement-dependent cytotoxicity). These limitations of mouse antibodies have largely been overcome by their chimerization, or humanization, or by the production of fully human antibodies [26], and such advances have ushered in the current era of antibody therapeutics. In the context of this scenario, Queen et al. also generated a humanized version of the anti-Tac mAb (daclizumab) [27]. In cell lines, it was thought that daclizumab blocked the interaction of IL-2 and its receptor, resulting in apoptotic cell death. Clinical efficacy in ATL was achieved predominantly in patients with smoldering or chronic ATL. Responding patients were hypothesized to have a blockage of the IL-2–IL-2 receptor autocrine pathway. Unlike other therapeutic mAb used in the treatment of human malignancies, daclizumab did not seem to have a strong cytocidal activity [24].

7.2 Radionuclide/toxin-conjugated CD25 targeting therapy

The limited efficacy of unmodified anti-CD25 antibody therapy prompted investigators to develop an alternative approach to enhance antitumor activity, i.e., using this agent as a carrier of radionuclides or toxins. Waldmann et al. [28] administered 5, 10, 15 mCi Yttrium-90-conjugated anti-Tac to 18 ATL patients, and 9 (7 PR and 2 CR) of 16 (56%) evaluable patients responded. Grade 3 and more severe therapy-related adverse events, such as granulocytopenia and thrombocytopenia, were limited to the hematopoietic system, however, a significant fraction of patients also developed HAMA, thus preventing the ability to administer repeated treatments. A Pseudomonas exotoxin A-single chain anti-CD25 (sc) Fv-fragment fusion protein (LMB-2) showed promising results in IL-2 receptor α-expressing lymphoid malignancies, including ATL [29]. A phase II trial of LMB-2 combined with fludarabine and cyclophosphamide for ATL is currently ongoing (ClinicalTrials.gov: NCT00924170). Other approaches to CD25 receptor-targeted therapy use ligand–toxin fusion molecules. Denileukin diftitox (Ontak) is a recombinant fusion protein composed of diphtheria toxin-A and a human IL-2 fragment [30]. It is able to direct its cytotoxic action to cells expressing a functional IL-2 receptor, and when it is internalized, active diphtheria toxin is released to kill tumor cells. In a pivotal trial in 71 patients with advanced refractory cutaneous T-cell lymphoma (CTCL), Ontak produced a 30% response rate with 10% CRs [31], while this study did not include ATL patients. Now, a phase II study of the efficacy and toxicity of Ontak in the therapy of ATL is ongoing (ClinicalTrials.gov: NCT00117845).

8 CC chemokine receptor 4 (CCR4)-targeting antibody therapy for ATL

8.1 ATL and CCR4

Chemokines are a group of structurally related small cytokines (8–14 kDa) that play essential roles in migration and homing of lymphocytes. Individual T-cell subsets express specific sets of chemokine receptors that impart unique migration and tissue homing properties to the cells [32], so manipulating a specific chemokine network can lead to altered behavior of the corresponding functional T-cell subset. CCR4 is known to be expressed selectively on regulatory T (Treg) and type 2 helper T (Th) cells [33–37] (Fig. 1). Yoshie et al. first carried out RT-PCR analysis of CCR4 in peripheral blood mononuclear cells (PBMCs) from ATL patients, and observed strong signals in 22 out of 24 patients (92%). They also showed that most ATL cases were positive for CCR4 by flow cytometric analysis (8/10, 80%) and immunologic staining of blood smears (6/7, 86%) [38]. We also performed an extensive immunohistochemical study of CCR4 on tissue biopsy samples from 103 ATL patients, and then reported that tumor cells from a large majority of patients with ATL expressed CCR4 (91/103, 88.3%) [39].

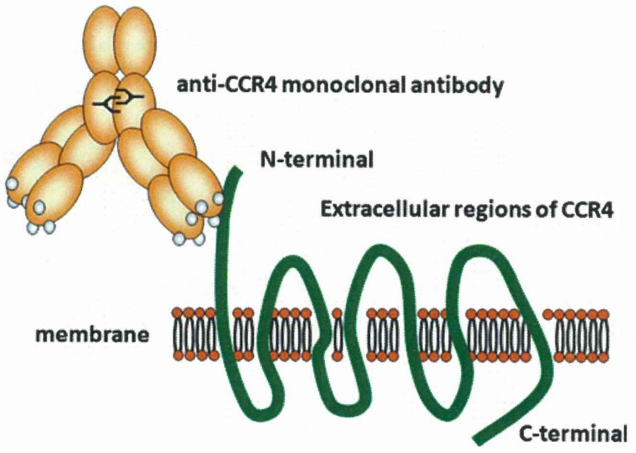

Fig. 1 CC chemokine receptor 4 (CCR4) and humanized anti-CCR4 monoclonal antibody, KW-0761. The *CCR4 gene* is located on chromosome 3p24. CCR4 is a seven-domain transmembrane G protein-coupled receptor, and TARC/CCL17 and MDC/CCL22 are ligands of CCR4. The anti-CCR4 monoclonal antibody recognizes the N-terminal portion of the CCR4 molecule

8.2 Importance of ADCC in antibody therapy

ADCC is triggered following binding of the antibody Fc region to the Fcγ-receptor (FcγR) on effector cells, and is believed to represent the major in vivo antitumor mechanism of the antitumor therapeutic mAbs such as rituximab, trastuzumab, and alemtuzumab [40–42]. In particular, Carton et al. [42] found that follicular lymphoma patients homozygous for FcγRIIIa-158VV, which has the highest affinity for the IgG1 Fc region, have the best clinical and molecular responses to rituximab. The clear role for FcγR-bearing effectors in mediating the response to rituximab in clinical settings further demonstrates the importance of ADCC.

8.3 ADCC-enhancing defucosylated mAb

ADCC requires the presence of oligosaccharides in the Fc region and is sensitive to change in the oligosaccharide structure [43]. Of all the sugar components in the oligosaccharide, galactose, bisecting-GlcNAc, and fucose [44, 45] have been reported to affect ADCC. Shinkawa et al. [45] reported that fucose was the most important of these sugar components, and that defucosylation of IgG1 enhanced their ADCC >50-fold. The influence of non-fucosylated oligosaccharide on ADCC has also been reported by Shields et al. [44]. Subsequently, we revealed that fucose depletion of IgG1 could reduce the antigen amount on target cells required for the same level of ADCC induction by 3- to 10-fold [46]. Recently, it has been generally accepted that endogenous plasma IgG inhibits ADCC mediated by the therapeutic mAb by a competition for binding to FcγIIIa on effector cells, especially NK cells [47]. Iida et al. [48] reported that defucosylated IgG1 can evade the inhibitory effect of plasma IgG on ADCC through its high level of FcγRIIIa binding. Defucosylated IgG1 also has increased binding affinity to FcγRIIIb on neutrophils, leading to enhanced antibody-dependent neutrophil phagocytosis [49].

8.4 Preclinical study of the therapeutic defucosylated anti-CCR4 mAb

The work demonstrating that CCR4 was expressed on tumor cells from most ATL patients prompted us to postulate that this molecule might represent a novel molecular target for mAb therapy in ATL. Together with the ADCC-enhancing defucosylation technology, the therapeutic chimeric (KM2760) and humanized (KW-0761) anti-CCR4 mAbs were developed [50, 51] (Fig. 2). The therapeutic defucosylated anti-CCR4 mAb induced a robust ADCC activity against not only CCR4-positive human ATL cells, but also against other types of CCR4-positive human leukemia/lymphoma lines in the presence of PBMC from healthy individuals in a dose-dependent manner in vitro. In addition, a robust ADCC of the therapeutic defucosylated anti-CCR4 mAb mediated by autologous effector cells was triggered in some ATL cells as well as cells from other types of PTCL patients in vitro [52, 53].

Next, we evaluated antitumor activity of this antibody in mice in vivo, and this mAb demonstrated a significant antitumor effect in disseminated and non-disseminated CCR4-positive lymphoma models in SCID mice [54, 55]. The defucosylated anti-CCR4 mAb can induce a highly enhanced ADCC activity, but it does not mediate complement-dependent cytotoxicity or possess direct antitumor activities [51, 52]. ADCC depends on the cytotoxic activity of immune effector cells, so to evaluate the antitumor effects of therapeutic mAb in a small animal model in vivo, the species incompatibility of the immune systems in humans and animals is a critical issue. Indeed, we have previously reported that the mouse effector system mediating the antitumor action of therapeutic mAb does differ from that of the human [54, 55]. Thus, a current crucial problem in the field of human ADCC research is the lack of suitable small animal models. To overcome this, we have recently established "humanized mice," in which human immune cells mediate the antitumor action of the therapeutic mAb, using NOD/Shi-*scid*, IL-2Rγnull (NOG) mice [56, 57] as recipients. In this model, we showed that human PBMC from healthy individuals functioned as ADCC effector cells against allogeneic tumor cell lines engrafted in the mice [58]. Using this humanized mouse model, we had the opportunity to perform more appropriate preclinical evaluations of many types of Ab-based immunotherapy, although in the initial study we could not completely

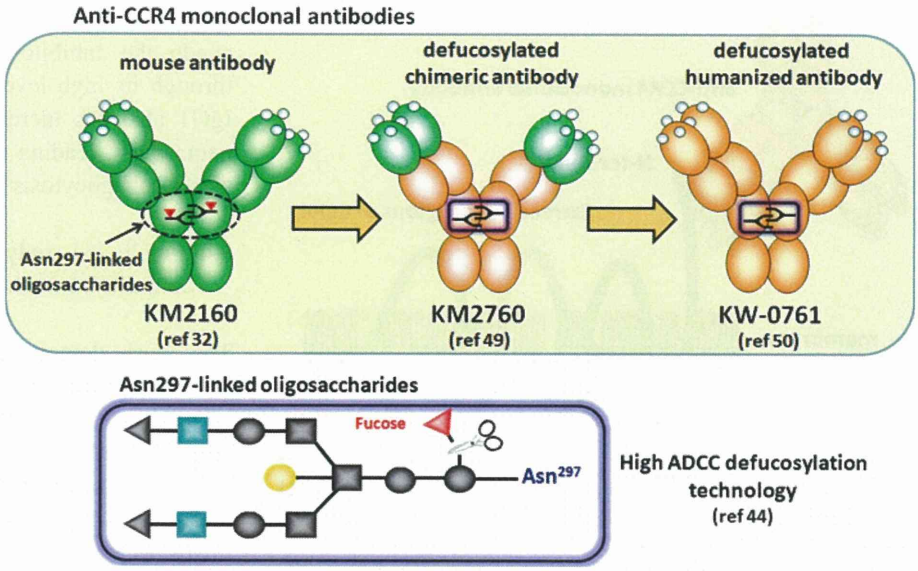

Fig. 2 Development of the therapeutic anti-CCR4 monoclonal antibodies. The structure of oligosaccharide in the Fc region of human IgG1 is shown. Antibody-dependent cellular cytotoxicity (ADCC) requires the presence of oligosaccharides in the Fc region and is sensitive to change in the oligosaccharide structure. Of all the sugar components in the oligosaccharide, fucose has the most important influence on ADCC. The therapeutic anti-CCR4 mAbs, KM2760 and KW-0761, whose Fc regions are defucosylated were developed to enhance ADCC activity by increasing its binding affinity to the Fc receptor on effector cells

exclude nonspecific allogeneic immune responses because target and effector cells were obtained from different individuals. To overcome these problems, we have established a primary human-tumor-bearing NOG mouse model, in which autologous human immune cells are engrafted and mediate ADCC, and endogenous murine cells are unable to mediate ADCC. In that study, we used primary ATL cells-bearing NOG mice. The therapeutic anti-CCR4 mAb showed significant antitumor activity against the primary ATL cells in the NOG mice in vivo by a robust ADCC mediated by autologous effector cells [59]. That study was the first to report on a mouse model in which a potent antitumor effect of the therapeutic mAb against primary tumor cells was mediated by autologous human immune cells. This approach should make it possible to model a human immune system that is active in mAb-based immunotherapy in vivo, and thus to carry out more appropriate preclinical evaluations of novel therapeutic mAbs.

8.5 Clinical studies of KW-0761

Based on the promising results of this preclinical work, KW-0761 moved to the clinical phase. Yamamoto et al. [60] reported the results of a multicenter phase I study of KW-0761 in Japan (Clinicaltrials.gov: NCT00355472) in patients with relapsed CCR4-positive T-cell leukemia/lymphoma, including 13 ATL, 2 PTCL-not otherwise specified, and a mycosis fungoides. This phase I study was the first clinical trial to examine the safety and efficacy of a next-generation defucosylated therapeutic antibody against cancer. The study demonstrated good tolerability and predictable pharmacokinetics, and resulted in a recommended dose of 1.0 mg/kg for subsequent clinical trials. This study also demonstrated preliminary evidence of potent antitumor activity, thus, objective responses were observed in 31% of patients, with 13% CRs. Among 13 ATL patients, objective responses were observed in 31% of patients, with 15% CRs. In this study, clinical responses were observed even at 0.01 mg/kg, which is approximately 1/1,000 of the rituximab dose of about 10 mg/kg (375 mg/m^2) [18, 19]. The clinical effect observed at the 0.01 mg/kg dose of KW-0761 would be consistent with the concept of using defucosylation of therapeutic mAbs to enhance ADCC [45].

We subsequently carried out a multicenter phase II study of KW-0761 in patients with relapsed, aggressive CCR4-positive ATL in order to evaluate efficacy, pharmacokinetic profiles, and safety. The patients received 8 weekly intravenous infusions of KW-0761 at doses of 1.0 mg/kg. Objective responses were noted in 13 of 26 evaluable patients, including 8 CRs, with an overall response rate of 50% (95% CI 30–70%) (Ishida et al., ASH Annual Meeting Abstracts 2010 116: Abstract 285). The median progression-free and OS were 5.2 and 13.7 months, respectively. These results suggested an improvement compared to the results achieved with other agents in relapsed ATL [61]. The most common adverse events were infusion reactions and skin rashes, which were manageable and reversible in all cases. Collectively, this phase II study demonstrated that KW-0761 showed clinically meaningful antitumor activity in patients with relapsed ATL, with an acceptable toxicity profile. Based on this phase II study, an application has been submitted for approval of KW-0761 use in relapsed CCR4-positive ATL to Pharmaceuticals and Medical Devices Agency (PMDA), Japan, in April, 2011.

Following upon a phase II study, JCOG9801, on untreated aggressive ATL [5], the present promising results of KW-0761 monotherapy prompted us to conduct a subsequent randomized trial of VCAP-AMP-VECP chemotherapy with or without KW-0761 for previously untreated ATL (Clinicaltrials.gov:NCT01173887). CCR4 is also expressed on

tumor cells from a subgroup of PTCL, other than ATL, which also has an unfavorable prognosis [62, 63]. Thus, we are currently conducting a phase II study of KW-0761 monotherapy in relapsed CCR4-positive PTCL (Clinicaltrials.gov: NCT01192984). In addition, Duvic et al. recently reported a phase I/II study of KW-0761 in refractory CTCL. They found that KW-0761 was well tolerated at doses of 0.1–1.0 mg/kg, and a promising overall response rate of 39% (15/38) was achieved, though expression of CCR4 on lymphoma cells was not included as one of the eligibility criteria (ASH Annual Meeting Abstracts 2010 116: Abstract 962, Clinicaltrials.gov: NCT00888927). The history of translational research on the anti-CCR4 mAb is shown in Fig. 3.

8.6 Possible future direction of KW-0761

It has been generally accepted that an increased concentration of Treg cells in the tumor microenvironment plays an important role in tumor escape from host immunity in several different types of cancer. Moreover, Treg cells infiltrating the tumor may represent one of the main obstacles to successful tumor immunotherapy. Therefore, depletion of Treg cells in the vicinity of tumors is a potentially promising strategy for boosting tumor-specific immunity [64–66]. We showed that the therapeutic anti-CCR4 mAb actually depleted Treg cells in vitro [36, 52], and, furthermore, that it also had this activity in vivo in humanized mice [58]. Collectively, these data suggest that therapeutic defucosylated anti-CCR4 mAb could also be used in novel strategies for treatment of many types of cancer to overcome the suppressive effect of CCR4-expressing Treg cells on the host's immune response to tumor cells. Simultaneously, attention should be paid to the immune-related adverse events caused by KW-0761, as observed in the anti-CTLA4 mAb, ipilimumab, study [67].

9 Other antibody therapies for ATL

A clinical trial of anti-CD52 antibody (alemtuzumab) for ATL has been conducted, but the obtained information is currently limited (Clinicaltrials.gov: NCT00061048). The antitubulin agent monomethyl auristatin E (MMAE) was attached to a CD30-specific mAb by an enzyme-cleavable linker, thus producing the antibody–drug conjugate brentuximab vedotin (SGN-35). Brentuximab vedotin induced durable objective responses and resulted in tumor regression in most patients with relapsed or refractory CD30-positive lymphomas [68]. Although ATL patients were not included in this study, a subgroup of ATL is positive for CD30 [39], thus brentuximab vedotin might be worth trying in such subgroups of ATL.

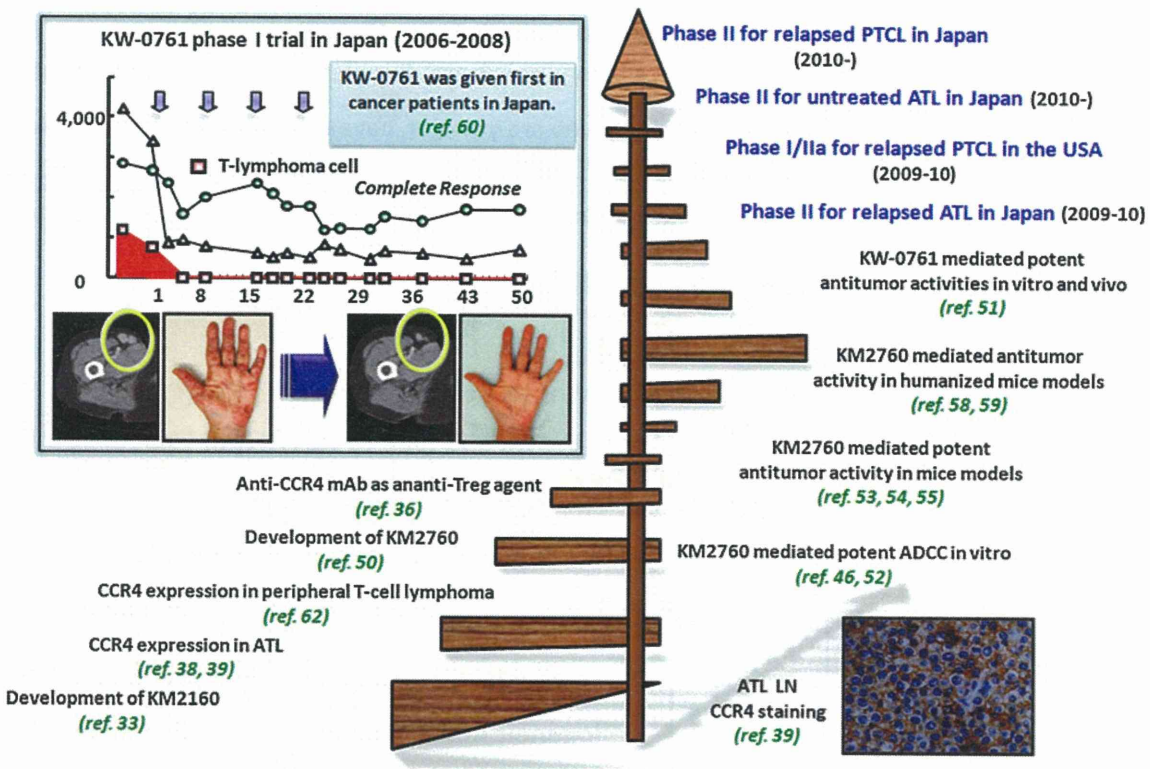

Fig. 3 Translational research of the anti-CCR4 mAb. Based on the preclinical studies—such as CCR4 expression in primary ATL cells, potent antitumor activity of the anti-CCR4 mAb against ATL cells in vitro and in mice in vivo—clinical trials of KW-0761 in ATL/PTCL were started

10 Future directions for care-giving of HTLV-1 carriers and treatments of ATL patients

Finally, we would like to discuss the possible future directions for care-giving of HTLV-1 carriers and treatments of ATL patients. HTLV-1 is transmitted mainly from mother to infant through breast milk, and then only a small subpopulation of the virus-infected individuals (approximately 5% of subjects) progresses to ATL after a long latency period of about 50–70 years [69]. Although we now have only few ideas of what kind of HTLV-1-infected individuals develop ATL, a recent cohort study of 1,218 asymptomatic HTLV-1 carriers demonstrated that high proviral load levels (especially >4 copies/100 PBMCs), advanced age, family history of ATL, and having first learned about the HTLV-1 infection during treatment of other diseases were independent risk factors for progression from carrier status to ATL [70]. In the context of this scenario, the establishment of prevention strategies in ATL development in such high risk HTLV-1 carriers is expected (Fig. 4, ①). Although, at the present time, smoldering or favorable chronic type ATL patients should be considered for watchful waiting or AZT/IFN therapy [71], the establishment of more effective treatment options leading to inhibition of the development of aggressive ATL in such indolent ATL patients is also expected (Fig. 4, ②③). It is now generally accepted that conventional chemotherapy alone seems to have reached the limits of efficacy and

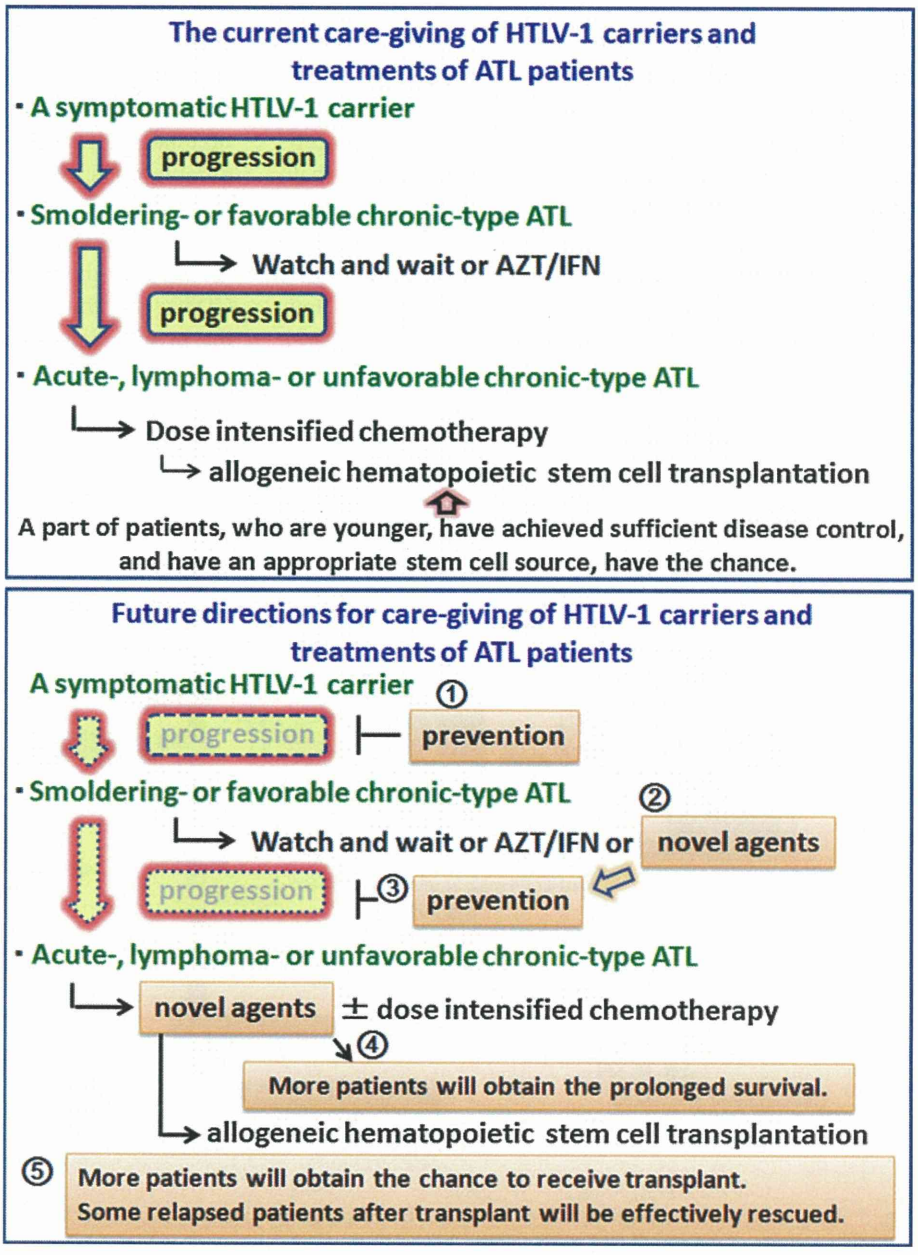

Fig. 4 Future directions for care-giving of HTLV-1 carriers and treatments of ATL patients. The current and future directions for care-giving of HTLV-1 carriers and treatments of ATL patients are described

presents an extremely low prospect for cure in patients with aggressive ATL [68]. Therefore, there is an urgent need for development of ideal treatment strategies—applied as monotherapy or combined with the current conventional chemotherapy—which will offer a more prolonged survival and lead to a complete cure of some patients with aggressive ATL (Fig. 4, ④). These novel treatments should simultaneously increase the percentage of ATL patients who are in sufficient disease control—possibly leading to an improvement in allogeneic hematopoietic stem cell transplantation for ATL—and rescue some patients who have relapsed after transplantation (Fig. 4, ⑤). Clinical applications of novel treatment strategies, including those involving KW-0761 and other promising antibody therapies mentioned in this paper, will make a contribution at each stage of the disease, from HTLV-1 carrier status to aggressive ATL, and provide a more favorable prognosis.

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Conflict of interest Takashi Ishida has received honoraria for lectures from Kyowa Hakko Kirin. Nagoya City University Graduate School of Medical Sciences has received research grant support from Kyowa Hakko Kirin for works provided by Takashi Ishida. No other conflict of interest relevant to this article was reported.

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