

Natural history of adult T-cell leukemia/lymphoma and approaches to therapy

Graham P Taylor¹ and Masao Matsuoka^{*2}

¹Department of GU Medicine & Communicable Diseases, Faculty of Medicine, Imperial College, Norfolk Place, London W2 1PG, UK; ²Laboratory of Virus Immunology, Center for AIDS Research, Institute for Virus Research, Kyoto University, 53 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

After cell-to-cell transmission, HTLV-I increases its viral genome by *de novo* infection and proliferation of infected cells. Proliferation of infected cells is clonal and persistent *in vivo*. During the carrier state, infected cells are selected *in vivo* by the host's immune system, the genetic and epigenetic environment of proviral integration sites, and other factors. In leukemic cells, *tax* gene expression is frequently impaired by genetic and epigenetic mechanisms. Such loss of *Tax* expression enables ATL cells to escape the host immune system. On the other hand, ATL cells acquire the ability to proliferate without *Tax* by intracellular genetic and epigenetic changes. Despite advances in support and the development of novel treatment agents, the prognosis for ATLL remains poor. A number of therapies, however, do appear to improve prognosis compared to CHOP (VEPA). These include interferon- α plus zidovudine (probably after 1–2 cycles of CHOP), intensive chemotherapy as in LSG-15 with G-CSF support and Allo-SCT (which includes the potential for cure). Emerging novel approaches include HDAC inhibitors, monoclonal antibodies, and proteasome inhibitors. Comparison between different therapeutic approaches is complicated by the range of natural history of ATLL, different recruitments of naïve-to-therapy, refractory or relapsed patients, and variations in the reporting of outcome that frequently excludes difficult-to-evaluate patients. Moreover, results from relatively small proof-of-principle studies have not been extended with randomized, controlled trials. As a result, currently, there is no clear evidence to support the value of any particular treatment approach over others. To avoid further unnecessary patient suffering and to identify optimal therapy as rapidly as possible, large randomized, controlled trials encompassing multicenter, international collaborations will be necessary.

Oncogene (2005) 24, 6047–6057. doi:10.1038/sj.onc.1208979

Keywords: ATLL; HTLV-I; *Tax*; chemotherapy; transplantation; prevention

Disease course from HTLV-I infection to onset of ATL

After infection by HTLV-I, a subpopulation of carriers (6% male and 2% female subjects) develops ATL after a long latent period. Although genetic, environmental, and viral factors in addition to the host immune response should be implicated in leukemogenesis, the exact mechanism remains to be elucidated. Below, we highlight first the current understanding of the disease course leading to the onset of ATL.

Transmission of HTLV-I

HTLV-I can infect various cell types, including T cells, B cells, and synovial cells, suggesting that its receptor is ubiquitously expressed. The receptor for HTLV-I has been identified as glucose transporter type 1 (GLUT1) (Manel *et al.*, 2003). Its expression on T lymphocytes is enhanced by mitogen or TGF- β (Jones *et al.*, 2005), which has been shown to increase the infectivity of HTLV-I. *In vitro* experiment shows that transmission by free virion is very inefficient, whereas transmission by infected cells is much more efficient. This is because HTLV-I transmits naturally in a cell-to-cell fashion. When an HTLV-I-infected cell attaches to uninfected cells, the HTLV-I-infected cells form 'virological synapses' with uninfected cells. Contact between an infected cell and a target cell polarizes the microtubule-organizing center (MTOC) at the cell–cell junction, and then viral proteins, such as Gag and viral genome RNA, accumulate at this junction and the viral complex subsequently transfers into the target cell (Igakura *et al.*, 2003). In HTLV-I-infected cells, expression of ICAM-1 is upregulated, and antibody to ICAM-1 induces MTOC polarization in HTLV-I-infected cells, suggesting that increased expression of ICAM-1 facilitates cell-to-cell transmission of HTLV-I (Barnard *et al.*, 2005). Thus, transmission of HTLV-I needs living infected cells.

HTLV-I-infected cells enter into the human body via three major routes: (1) mother-to-infant transmission (mainly through breast-feeding), (2) sexual transmission, and (3) parenteral transmission. Of note, fresh frozen plasma from seropositive donors did not transmit HTLV-I (Okochi *et al.*, 1984). Hence, if living cells can be eliminated by freeze and thawing, then feeding

*Correspondence: M Matsuoka;
E-mail: mmatsuok@virus.kyoto-u.ac.jp

the mother's breast milk to infants will not increase viral transmission (Ando *et al.*, 2004). These findings clearly show that physiological transmission requires live HTLV-I-infected cells. To facilitate its transmission, HTLV-I increases clonally the population of infected cells by the pleiotropic actions of viral proteins, especially Tax (Yoshida, 2001; Gatza *et al.*, 2003; Jeang *et al.*, 2004; Matsuoka and Jeang, 2005).

HTLV-I-infected cells *in vivo*

In HTLV-I carriers and ATL patients, no free virion has been demonstrated *in vivo*. The presence of antibody and provirus are evidence for HTLV-I infection. After transmission of HTLV-I, reverse transcriptase generates proviral DNA from the genomic viral RNA, and the provirus is integrated into the host genome by viral integrase. Since most ATL cells contain one copy of HTLV-I provirus, and ATL cells are derived from HTLV-I-infected cells, it is reasonable to consider that most of the HTLV-I-infected cells have one copy of the provirus. Therefore, quantification of provirus is thought to reflect the number of HTLV-I-infected cells *in vivo*. Quantification of HTLV-I provirus among infected individuals demonstrates that provirus load differs more than 1000-fold among asymptomatic carriers (Etoh *et al.*, 1999). Since living HTLV-I-infected cells are essential for transmission as mentioned above, an increased number of HTLV-I-infected cells is thought to facilitate transmission. Indeed, higher provirus load in the breast milk is correlated with an increased risk of vertical transmission from seropositive mothers (Li *et al.*, 2004). In this regard, the mechanism to increase HTLV-I-infected cells by actions of accessory genes, especially *tax*, provides the rationale for enhancing infectivity.

Clonal proliferation of HTLV-I-infected cells

After transmission, HTLV-I increases viral copy number both by *de novo* infection and clonal proliferation of infected cells. In this strategy, Tax plays a central role in increasing the number of HTLV-I-infected cells by promoting proliferation and inhibiting apoptosis. Since the integration sites of HTLV-I provirus are random, the demonstration of discrete integration sites can be employed to identify individual HTLV-I-infected clones. Inverse PCR or linker-mediated PCR was utilized to characterize integration sites, and revealed that proliferation of HTLV-I-infected cells was oligoclonal (Figure 1) (Etoh *et al.*, 1997). Importantly, these clonal proliferations of HTLV-I-infected cells are persistent since the same clones can be detected at the different time points (Etoh *et al.*, 1997; Cavrois *et al.*, 1998). As an example, a HAM/TSP patient developed lymphoma-type ATL. The ATL clone was identified in a blood sample obtained before the onset of ATL, which showed that the same clone was already present during HAM/TSP (Tamiya *et al.*, 1995). In addition, the prospective study of the Miyazaki cohort identified carriers who developed ATL during follow-up. The presence of

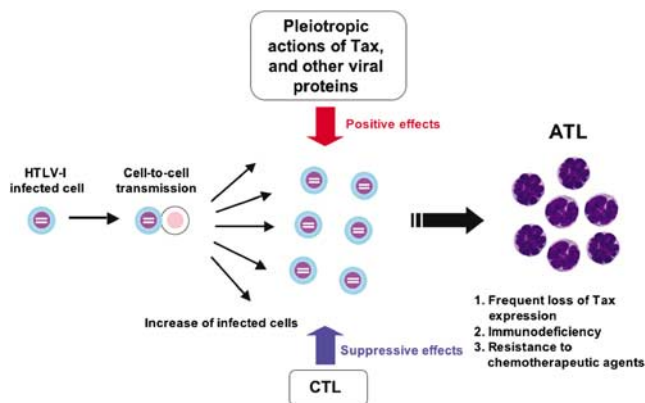


Figure 1 Natural course from HTLV-I infection to onset of ATL. HTLV-I is transmitted in a cell-to-cell fashion. After infection, HTLV-I promotes clonal proliferation of infected cells by pleiotropic actions of Tax and other viral proteins. Proliferation of HTLV-I-infected cells is controlled by cytotoxic T cells *in vivo*. After a long latent period, ATL develops in about 5% of asymptomatic carriers. The expression of Tax is inactivated by several mechanisms, suggesting that Tax is not necessary in this stage. Alternatively, alterations and errors in the host genome accumulate progressively during the latent period, finally leading to onset of ATL.

leukemic clones was detected during the carrier state by inverse PCR method. Such clonal proliferation is directly associated with the onset of ATL (Okayama *et al.*, 2004), and these studies clearly illustrate that HTLV-I-infected clones can transform to malignancy during the carrier state.

HTLV-I clonal cells are more heterogeneous and less stable during seroconversion than in long-term carriers (Tanaka *et al.*, 2005). This finding suggests that during seroconversion, HTLV-I-infected cells are actively selected *in vivo*, and thereafter, selected clones predominate in long-term carriers. Such selection may be due to several factors, including the immune pressure exerted by cell-mediated immunity (Bangham, 2003), the productivity of viral proteins, and the characteristics of the integration sites which influence viral transcription.

Integration sites of HTLV-I provirus

To clarify the significance of integrated genome positions, integration sites of HTLV-I provirus are determined by inverse PCR in carrier states and ATL. The frequencies of HTLV-I provirus integration into transcription units (from the first exon to the last exon) are 26.8% (15/56) in carriers and 33.9% (20/59) in ATL. These are equivalent to the frequency calculated based on random integration (33.2%) (Doi *et al.*, 2005). By contrast, HIV-1, another human retrovirus, integrates predominantly (91%) into actively transcribed genes of T lymphocytes (Han *et al.*, 2004), and integration sites are evenly distributed within the transcription units. Additionally, the frequency of integration into transcription units is 34.2% for murine leukemia virus (MLV), and these sites are clustered near the transcriptional

start sites (Wu *et al.*, 2003). Similarly, HTLV-I provirus is prone to integration near the transcriptional start sites in leukemic cells ($P = 0.006$). Thus, integration pattern of HTLV-I is similar to MLV rather than HIV-1. HIV-1 can infect nondividing cells by passing through the nuclear pore, whereas MLV can infect only dividing cells. These data suggest that HTLV-I may infect only dividing cells.

In addition, HTLV-I integration sites in the carriers favor the alphoid repetitive sequences (11/56: 20%) whereas in leukemic cells they disfavored these sequences (2/59: 3.4%). Alphoid repeats are a component of centromeric heterochromatin and consist of monomeric 171 bp repeats. When HIV-1 is integrated into alphoid repetitive sequences, it adopts a latent state due to the influence of the surrounding heterochromatin (Jordan *et al.*, 2003). Thus, genome surrounding integration sites influences the transcription of viral genes. HTLV-I-infected cells in which the provirus is integrated into alphoid repetitive sequence are considered to be in the latent state. Such infected cells are enriched in carrier state, indicating that infected cells with less viral gene expression are favored since such cells can escape from the host's cytotoxic T lymphocytes (Bangham, 2003). However, integration into alphoid sequences is infrequent in leukemic cells, indicating that among surviving HTLV-I-infected cells those with higher production of viral proteins are more likely to transform into malignant cells.

HTLV-I provirus in ATL cells

The *tax* gene plays a central role by its pleiotropic actions in the proliferation and leukemogenesis of HTLV-I-infected cells *in vivo* (Franchini *et al.*, 2003). However, its transcription is detected in only 34% of ATL cases by RT-PCR (Takeda *et al.*, 2004). Tax production is impaired by several mechanisms: (1) genetic changes (nonsense mutation, insertion, and deletion) of *tax* gene, (2) deletion of 5'-long terminal repeat (LTR), and (3) DNA methylation of 5'-LTR. Genetic changes of *tax* gene were observed in five of 47 cases (11%) (Furukawa *et al.*, 2001; Takeda *et al.*, 2004). Deletion of 5'-LTR, which is the promoter/enhancer for viral gene transcription, was observed in 14 of 47 cases (30%). This type of defective provirus designated type 2 defective provirus (Tamiya *et al.*, 1996) lacks 5'-LTR and internal sequences, such as *gag*, *pol*, and *env* genes. The frequency of type 2 defective provirus was much higher in acute and lymphoma-type ATL than in chronic type ATL, suggesting a close association with disease progression. Heavy methylation of the 5'-LTR region was also associated with silencing of viral gene transcription (Koiwa *et al.*, 2002; Takeda *et al.*, 2004). Tax expression is absent or severely impaired in ATL cells with heavily methylated 5'-LTR. Such changes are predominantly observed in aggressive subtypes of ATL, which suggests that at this stage, ATL cells acquire the ability to proliferate without Tax expression. In turn, such changes enable ATL cells to escape from the host immune system by

loss of Tax expression (Figure 1). This finding reveals a duality in the Tax protein: its expression induces proliferation and inhibits apoptosis of HTLV-I-infected cells, and also evokes the host's immune response including cytotoxic T cells to kill virus-infected cells.

Somatic changes in ATL cells

As described above, ATL cells do not always need Tax expression in the later stage of leukemogenesis. Genetic and epigenetic changes imprinted into the genome should be implicated in such multistep leukemogenesis. Regarding genetic changes, mutation of p53, and deletion of p16 have been reported in ATL. Usually, ATL cases with genetic changes in p53 and p16 have a poor prognosis (Yamada *et al.*, 1997). Therefore, these genetic changes are thought to be associated with disease progression. A transcriptional profile of ATL cells by DNA chip analysis identified aberrantly transcribed genes (Sasaki *et al.*, 2005). Among them, the expression of *tumor suppressor in lung cancer 1 (TSLC1)* gene is upregulated in ATL cells. *TSLC1* gene was identified as a tumor suppressor gene in lung cancer cells. Since this molecule is associated with cell adhesion, loss of its expression is associated with the invasive phenotype of lung cancer cells (Kuramochi *et al.*, 2001). However, its ectopic expression is associated with leukemogenesis possibly due to the conferring of an adhesive phenotype to ATL cells.

Epigenetic changes are recognized as mechanisms implicated in oncogenesis as well as genetic changes. Since genetic changes in specific genes in ATL cells have not been identified except for p53 and p16, and there is no consistent chromosomal change, it is possible that epigenetic changes such as DNA methylation play an important role in leukemogenesis by inhibiting the transcription of tumor suppressor genes or inducing aberrant expression of oncogenes. Aberrantly methylated DNA regions were identified by a methylated CpG-island amplification/representational difference analysis method (Toyota *et al.*, 1999). *MELIS* gene was hypomethylated and aberrantly expressed in ATL cells (Yoshida *et al.*, 2004). Since such expression conferred the resistance against TGF- β , the *MELIS* gene expression is implicated in resistance of ATL cells to TGF- β . On the other hand, *EGR3* gene has been demonstrated to be hypermethylated in ATL cells (Yasunaga *et al.*, 2004). *EGR3* is a transcriptional factor, which is essential for transcription of the *FasL* gene (Mittelstadt and Ashwell, 1998). Normal activated T lymphocytes express FasL as well as Fas antigen. Apoptosis induced by autocrine mechanisms is designated activation-induced cell death (AICD) and this controls the immune response (Krueger *et al.*, 2003). Although ATL cells express Fas antigen, they do not produce FasL, thereby enabling ATL cells to escape from AICD. Suppressed transcription of *EGR3* gene might be the mechanism allowing ATL cells to escape from AICD. Thus, epigenetic changes of the genome play an important role in oncogenesis of ATL.

Pathogenesis

Hypercalcemia

Hypercalcemia complicates more than 70% of ATL cases during the entire clinical course (Kiyokawa *et al.*, 1987), and the extent of hypercalcemia is frequently severe. In the bone of hypercalcemic patients, the number of activated osteoclasts increases, which accelerates bone resorption. In the differentiation of osteoclast from the hematopoietic precursor cells, RANK ligand, which is expressed on the osteoblasts, and M-CSF cooperatively induce the differentiation of osteoclasts (Arai *et al.*, 1999). In hypercalcemic ATL patients, ATL cells have been shown to express RANK ligand (Nosaka *et al.*, 2002), and the serum level of M-CSF is elevated in most ATL patients. ATL cells from hypercalcemic patients have been demonstrated to induce the differentiation of hematopoietic precursor cells into osteoclasts *in vitro*. These data indicate that ATL cells expressing RANK ligand induce the differentiation to osteoclast, and such increased osteoclasts accelerate bone resorption, resulting in hypercalcemia. In ATL patients, parathyroid hormone-related peptide (PTH-rP) is frequently increased (Watanabe *et al.*, 1990), which induces the RANK ligand expression on osteoblasts. Increased PTH-rP is also implicated in ATL-associated hypercalcemia.

Immunodeficiency

Opportunistic infections are frequent complications in ATL patients, and impaired cell-mediated immunity has been identified as a causative basis of immunodeficiency. Pathogens of opportunistic infections include *Pneumocystis jiroveci*, cytomegalovirus, *Strongyloides stercoralis*, and a variety of fungi. Such infections are one reason for the poor prognosis, despite treatment, of ATL patients. Mild immunodeficiency is also seen in asymptomatic carriers (Katsuki *et al.*, 1987; Welles *et al.*, 1994). Here, a decreased number of naïve T-lymphocytes is proposed as a cause of immunodeficiency (Yasunaga *et al.*, 2001). CD4+, CD25+ T cells are reported to have immunoregulatory functions, and are called as regulatory T cells (Treg). Tregs have suppressive immune functions. Tregs express *forkhead box P3* (*FOXP3*) gene, which is a master gene of immunoregulatory functions (Fontenot and Rudensky, 2005). ATL cells show the phenotype of activated helper T cell (CD4+ and CD25+), suggesting that they are derived from a Treg cell. *FOXP3* gene transcription was detected in eight of 17 ATL cases (47%) (Karube *et al.*, 2004). Such Treg phenotype of ATL cells is considered to suppress the immune response, and may be implicated in the immunodeficiency. In addition to the FOXP3+ Tregs, antigen-induced IL-10 secreting Tr1 has been identified as another subset of Treg (Thompson and Powrie, 2004), which is FOXP3 negative. Although FOXP3 expression was not detected in about half of ATL cases, ATL cells were reported to produce IL-10

suggesting that FOXP3-negative ATL cells also have regulatory contribution.

Treatment of ATLL

After 28 years of the initial description of ATLL as a discrete clinical entity, this condition continues to carry a very poor prognosis. Recent reviews cite median survivals of less than 1 year despite advances in both chemotherapy and supportive care (Siegel *et al.*, 2001). The 6 months median survival of Japanese patients with ATLL reported in Shimoyama's (1992) overview does not significantly differ from experience in Europe a decade later (Taylor *et al.*, 2001). It is therefore hard to disagree with Yamada and Tomonaga's conclusion that the vast accumulation of knowledge in the molecular biology and oncogenesis of ATLL has yet to be translated into an improved prognosis (Yamada and Tomonaga, 2003). Yet, the array of therapeutic approaches tested over the past two decades is impressive. In this section, we review extant data and attempt to determine why so many therapies, initially reported optimistically, have not been further developed. We look to how prognosis might be improved in the future.

Chemotherapy

Cyclophosphamide, adriamycin, vincristine, and prednisolone (CHOP) has been, and probably remains, the standard first-line therapy for ATLL and many patients do exhibit either partial (PR) or complete remission (CR). Yet a literature search will reveal only limited data on the efficacy of this approach. Tsukasaki *et al.* reviewed the outcome of their cohort of 114 patients presenting with acute or lymphomatous ATLL between 1975 and 1989. These patients were treated with combination chemotherapy with only 17.5% achieving CR, but a further 46.5% had a partial response (Tsukasaki *et al.*, 1993). These data accord with the results of the Lymphoma Study Group in which CR was obtained in 17–18% of patients treated with CHOP (LSG-1) (Lymphoma Study Group, 1982; Shimoyama *et al.*, 1988); in 37% of patients treated with CHOP plus methotrexate (Shimoyama *et al.*, 1988). A similar response (63% CR+PR) was found in 21 patients treated with combination chemotherapy in London, UK over a 15-year period (1981–1995), but the median survival was only 5.5 months (Pawson *et al.*, 1998). Intensification of CHOP with etoposide, vindesine, ranimustine, and mitoxantrone resulted in CR in 35.8% of the 83 patients (Taguchi *et al.*, 1996) and in 43% when part of a nine-agent cycle (LSG-4) (Tobinai *et al.*, 1994). However, the median survival was only 8–8.5 months in these studies with predicted survivals of 13.5% after 3 years and 12% after 4 years, respectively. Matsushita *et al.* reported their experience of substituting etoposide for adriamycin in a weekly long-term maintenance chemotherapy. The median survival in their 79 patients with acute, lymphoma, and progressive

chronic ATLL following this regimen was 7.5 months, but the therapy was reported to be well tolerated (Matsushita *et al.*, 1999). Better survival, 18 months, was observed among a further eight patients treated with daily etoposide plus prednisolone, but this may have been due to selection bias. The best outcome with chemotherapy reported to date has been with an aggressive multidrug approach supported by G-CSF. While this regimen of seven cycles of VCAP (vincristine, cyclophosphamide, doxorubicin, and prednisone), AMP (doxorubicin, ranimustine, and prednisone), and VECF (vindesine, etoposide, carboplatin, and prednisone) was, as anticipated, highly marrow toxic with grade 4 haematological toxicity in the majority of patients, the median survival of 13 months among 96 treatment naïve patients with acute, lymphoma and progressive chronic ATLL does represent an improvement (Yamada *et al.*, 2001). The relative insensitivity of ATLL to chemotherapy may be related, at least in part, to upregulation of MDR gene (Kuwazuru *et al.*, 1990). In the Yamada study, ranimustine and carboplatin were included in the regimen because they are not affected by P-glycoprotein expression.

Part of the problem lies in the variable natural history of ATLL, and therefore the balance of disease types in the cohort may affect the outcome. For this reason, case reports are almost uninterpretable. In Uozumi's study, the median survival of 43 patients with acute and lymphoma ATLL treated with a response-orientated cyclic multidrug protocol was only 6 months, but many patients had poor prognostic factors (Uozumi *et al.*, 1995). While in Matsushita's cohort of maintenance therapy, the survival of patients with acute leukemia was 6.7 months, lymphoma 9.6, and progressive chronic leukemia 12.4 months (Matsushita *et al.*, 1999). It is difficult to judge whether this approach represents an advance in therapy as suggested by the authors although this remains a possibility. The best response rates are with the LSG-15 regime on which patients with acute ATLL survived 10.9 months and patients presenting with lymphoma 19.8 months (Yamada *et al.*, 2001). However, because renal dysfunction was an exclusion criterion, no patients with severe hypercalcemia were included in the study. This may have contributed to the superior results seen with this protocol. Overall, ATLL survival with various chemotherapy regimens is poor, with survival in several cohorts of patients presenting predominantly with acute leukemia or lymphoma ranging between 5.5 and 13 months. This approach does not offer the prospect of cure (Table 1).

Nucleoside analogues

A number of studies have addressed the role of nucleoside analogues in the management of ATLL. The purine analog 2' deoxycoformycin (DCF) that inhibits adenosine deaminase has been investigated as an alternative approach. In a phase I dose finding safety study, 3/18 patients with ATLL had a PR with 3 days intravenous (i.v.) therapy. The suggested dose for phase II trials was 5 mg/m² i.v. for 3 days (Tobinai *et al.*, 1992).

In another study using DCF 4 mg/m²/week for 4 weeks followed by fortnightly therapy, two CR and one PR were reported among 25 patients with ATLL (Mercieca *et al.*, 1994). While reported with a degree of optimism, these response rates are clearly lower than with CHOP-based chemotherapy. Using DCF in conjunction with chemotherapy, 52% of 60 patients achieved CR, but the median survival of all patients was only 7.4 months (Tsukasaki *et al.*, 2003).

Although one patient with treatment resistant acute ATLL had a prolonged partial response to another adenosine analog 2' chlorodeoxyadenosine (cladribine) (Uike *et al.*, 1998), the follow-up phase II study showed very limited benefit in 15 patients, with only the one, presumably same, response reported. However, all patients had been treated with other agents prior to entering this study and therefore represent a poor prognosis group (Tobinai *et al.*, 2003). Another purine (adenosine) derivative has been studied in phase I. Dose-limiting marrow toxicity was observed with fludarabine phosphate (Arima *et al.*, 1999).

A parallel approach, using L-alanosine, an inhibitor of adenosine monophosphate synthesis, has been suggested based on the observation of increased sensitivity of ATLL cells to L-alanosine *in vitro*. A proportion of ATLL primary cells are deficient in methylthioadenosine phosphorylase (MTAP) which should make them more sensitive to purine synthesis inhibitors. Nonleukaemic cells can, *in vitro*, be protected from this effect by simultaneous treatment with 5'-deoxyadenosine (Harasawa *et al.*, 2002). No *in vivo* data have been published to date and the therapy would need to be selectively used in MTAP-deficient ATLL only.

Topoisomerase inhibitors

Complete remission lasting 5 months after treatment with CPT-11, irinotecan hydrochloride, an inhibitor of topoisomerase I was reported in a patient with ATLL lymphoma unresponsive to intensified chemotherapy (Makino *et al.*, 1994). However in a study of 13 patients, all pretreated and failing chemotherapy, only one patient had a CR (Tsuda *et al.*, 1994). Although PR was seen in a further four patients, this agent has not been further studied in this group, in therapy naïve, nor in combination with other agents.

MST-16, a bis(2,6-dioxopiperazine) analog and inhibitor of topoisomerase II has been studied in a phase I–II trial (Ohno *et al.*, 1993). A total of 24 patients received 1200–2800 mg/day oral MST-16 for 7 days every 2–3 weeks. Remission occurred in both patients with chronic ATLL, 46% of patients with acute ATLL, but in only 25% of patients with lymphoma, and the two CR and eight PR lasted just over 2 months. These results do not represent an improvement over conventional chemotherapy and further studies with MST-16 have not been published.

Menogaril 100 mg daily 7 consecutive days every 3–4 weeks induced CR in 2/15 patients and PR in four (Taguchi *et al.*, 1997).

Table 1 Therapy studies inclusive of acute, lymphoma, and progressing or poor prognosis chronic ATLL

First author	Year	Therapy	Support	N	CR (%)	PR (%)	ALL (%)	Median survival (months)	Survival rate
Lymphoma Study Group	1982	CHOP (VEPA) aka LSG-1			18			6.0	
Shimoyama, M	1988	VEPA v		54	17			6.0	4 years 8%
Tobinai	1994	VEPA-M (aka LSG-2)			37			8.0	4 years 12%
		VEPA-B/M-FEPA/VEPP-B (aka LSG-4)			43				
Tsukasaki, K	1993	Combination chemotherapy (single institution cohort)		114	17.5	46.5	64		
Uozumi, K	1995	Response-orientated chemotherapy ?All treatment naïve		43	20.9	65.1	86	6.0	
Taguchi, H	1996	CHOP + etoposide, vindesine, ranimustine and mitoxantrone	G-CSF	83	35.5	38.3	74.1	8.5	3 years 13.5%
Pawson, R	1998	Combination chemotherapy		21			63	5.5	
Matsushita, K	1999	OPEC/MPEC or etoposide/Pred (maintenance)		79	31	58.6	89.6	7.5	
Yamada, Y	2001	VCAP/AMP/VECP (JCOG9309/LSG-15) all treatment naïve	G-CSF	96	35.5	45.2	81	13.0	2 years 31%
Tsuda, H	1994	CPT-II, topoisomerase-I inhibitor (refractory disease)		13	7.7	30.1	38.8		
Ohno, R	1993	MST-16, topoisomerase-II inhibitor		24	8.33	25.0	33.3		
Tsukasaki, K	2003	VPA/etoposide + deoxycoformycin aka LSG-11/JCOG9109		60	28	24	52	7.4	2 years 17%
Gill, P	1995	Interferon- α + zidovudine (half had refractory/relapsed disease)		19	26.3	31.5	57.8	3.0	
Hermine, O	1995	Interferon- α + zidovudine		18			66	10.0	
Matutes, E	2001	CHOP, interferon- α + zidovudine		15			67	18.0	
Hermine, O	2002	+/-CHOP, interferon- α + zidovudine		19	47	21	68	11.0	
Besson, C	2002	CHOP, interferon- α + zidovudine, etoposide		7				17.0	
Waldmann, T	1988	Anti-Tac antibody		19	11	21	33		
Waldmann, T	1995	90Y-labelled anti-Tac antibody		18	11	39	50		
Fukushima, T	2005	Allo-SCT		40	100			9.6	3 years 45.3%
<i>Acute ATLL only</i>									
Ohno, R	1993	MST-16, topoisomerase-II inhibitor		13	6.23	38.4	46.1		
Matsushita, K	1999	OPEC/MPEC or etoposide/Pred (maintenance)		51				6.7	
Yamada, Y	2001	VCAP/AMP/VECP	G-CSF	56	19.6	53.6	73.2	10.9	
Hermine, O	2002	+/-CHOP, interferon- α + zidovudine		15					
<i>Lymphoma only</i>									
Ohno, R	1993	MST-16, topoisomerase-II inhibitor		8	0	25.0	25.0		
Matsushita, K	1999	OPEC/MPEC or etoposide/Pred (maintenance)		22				9.6	
Yamada, Y	2001	VCAP/AMP/VECP	G-CSF	27	66.7	29.6	96.3	19.7	

Definitions of disease response (Yamada *et al.*, 2001): Complete remission – disappearance of all clinical and radiological evidence of disease and normalization of LDH for at least 4 weeks. Partial remission – greater than 50% reduction in disease for more than 4 weeks, and > 75% reduction in absolute abnormal lymphocyte counts, and LDH < 1.5 \times normal upper limit

All-*trans*-retinoic acid (ATRA), an analog of vitamin A induces G1 cell-cycle arrest and induction of apoptosis in *ex vivo* ATLL cells. Exposure of cell lines derived from ATLL patient PBLs to ATRA results in increased cyclin D1 protein and an increase in complex formation with cyclin-dependent kinases 4 and 6 (cdk4/cdk6) and with proliferating cell nuclear antigen (PCNA). The effects of ATRA on these cell lines are complicated with evidence of an initial increase in cdk2 activity followed by depression of activity. Thus

following ATRA, these cells were initially stimulated and then arrested in G1 (Dierov *et al.*, 1999).

Interferon

Although ineffective alone, interferon- α does have a role in the management of ATLL especially in combination with zidovudine. The first report of potential benefit was the study by Ezaki *et al.* (1991) in which 9/12 patients, some pretreated with chemotherapy, had a PR with

human lymphoblastoid interferon in combination with bestrabucil (a combination of chlorambucil with β -estradiol). However, the treatment of all patients with lymphoma or hypercalcemia with prednisolone makes this result more difficult to evaluate.

Zidovudine and Interferon plus zidovudine

The observation of clinical improvement in ATLL in a patient undergoing treatment for HIV-1 infection with zidovudine plus interferon- α led to the further investigation of this combination. In the USA, Gill *et al.* treated 19 patients, seven relapsing after chemotherapy, with zidovudine 200 mg \times 5/day plus interferon- α 5–10 MU s.c. daily, effecting CR in five and PR in six. Although four patients survived beyond 1 year, the median survival of only 3 months was indicative of the advanced disease in this cohort (Gill *et al.*, 1995). In France, Hermine *et al.* (1995) treated 18 patients with ZDV/IFN and achieved a 58% CR/PR response rate and a median survival of 10 months. In the UK, a debulking approach with 1–2 cycles of CHOP followed by a switch to lower doses of interferon- α (3–5 MU) plus zidovudine 500 mg bd was preferred. In total, 15 predominantly naïve, patients were treated in an open study, 67% achieving remission (CR + PR) with a median survival of 18 months (Matutes *et al.*, 2001). In a further phase II study from France of 12 treatment naïve patients with acute and lymphoma ATLL, the 92% response rate (seven CR and four PR) with zidovudine plus interferon- α represents a significant improvement over conventional and other chemotherapies (Hermine *et al.*, 2002). If all 19 patients, including the seven who did not receive ZDV/IFN first line, are included, the overall median survival remains a disappointing 11 months but 15/19 presented with the most aggressive, acute form of ATLL. Response and survival in patients who were treated with ZDV/IFN after initial chemotherapy was less impressive than those treated with ZDV/IFN as first-line therapy, but survival from initial presentation should also be considered. The Martinique experience, which is more similar to that of the UK, is to give two cycles of CHOP followed by ZDV/IFN (or sometimes the cytosine analog ddC instead of the thymidine analog ZDV) plus etoposide. The 17-month survival with this approach was significantly better than the historical survival of 3 months (Besson *et al.*, 2002).

Interferon- α and arsenic trioxide (As_2O_3)

In vitro studies have shown a synergistic effect of IFN- α and arsenic to induce apoptosis in ATLL cells. This combination was therefore offered to seven patients with refractory/relapsing ATLL in a pilot study. Although CR was seen in one patient persisting for a minimum of 56 months and PR in three patients, all patients had discontinued therapy after a median of 22 days due to toxicity or progression, and the six PR or unresponsive patients had died within a median of 1.5 months (Hermine *et al.*, 2004; Mahieux and Hermine, 2005). The median survival of these patients from first

presentation (6 months prior to As_2O_3) was, therefore, 7.5 months. Arsenic alone has been shown to block transcription of NF- κ B-dependent genes in HTLV-I-infected cells and in combination with IFN- α inhibits Tax-induced NF- κ B activation (Nasr *et al.*, 2003).

NF- κ B blockade

A number of apparently differing approaches point to the potential importance of NF- κ B activity in ATLL and the therapeutic potential of NF- κ B inhibition.

In vitro Bay 11-7082 inhibits NF- κ B, reduces DNA binding to NF- κ B, and downregulates transcription of Bcl- x_L . Preferential apoptosis of HTLV-I-infected cell lines and primary ATLL cells was observed. Unlike the histone deacetylation inhibitor, HFR901228, described below, Bay 11-7082 did not affect AP-1 (Mori *et al.*, 2002).

Inhibition of the proteasome by PS-341, bortezomib, blocking the degradation of I κ B α and thereby inhibiting NF- κ B has been shown to induce programmed death of ATLL cells *in vitro* (Tan and Waldmann, 2002; Satou *et al.*, 2004). Suppression of tumour growth was also documented in a SCID mouse ATLL model (Satou *et al.*, 2004). On the other hand, Tan and Waldmann (2002) reported no benefit in their mouse model of ATLL with PS-341 alone, but CR in some animals when combined with anti-Tac antibody.

The use of histone deacetylation inhibitors (HDIs) has recently attracted attention. One such compound, HFR901228, depsipeptide, has been shown to induce apoptosis in Tax-expressing and in Tax nonexpressing HTLV-I-infected cell lines and in primary cells for patients with acute ATLL. Its effect was through a reduction of DNA binding of NF- κ B and AP-1, and downregulation of Bcl- x_L and cyclin D2 expression. Partial inhibition of the growth of tumors, which result from the transplant of HTLV-I-infected cells, was seen in a SCID mouse model (Mori *et al.*, 2004). Sodium valproate, widely prescribed for the treatment of epilepsy, bipolar mood disorders, and migraine, has, among several potential antitumour properties, HDI activity (Blaheta and Cinatl, 2002). Sodium valproate is being studied as a maintenance therapy after chemotherapy for malignant glioma at a dose of 10–100 mg/kg/day. More importantly, dramatic clearance of both lymphoma and leukemia has been demonstrated in BLV-induced B-cell malignancy in sheep (Amine Achaichi, Arnaud Florins, Nicolas Gillet, Christophe Debacq, Patrice Urbain, Germain Manfouo Foutsop, Fabian Vandermeers, Agnieszka Jasik, Michal Reicher, Pierre Kerkhofs, Laurence Lagneaux, Arsene Burny, Richard Kettmann, and Luc Willems: submitted for publication). While a trial of this therapy as part of the management of patients with acute and lymphoma ATLL should be considered further, possibilities include prevention of progression of chronic and smouldering ATLL. Should a protective effect be shown, the long-standing safety profile of this compound would justify a study to prevent ATLL in patients at higher risk of disease. The profile of such patients, high anti-HTLV-I

antibody titer and high soluble IL-2 receptor levels, has been described (Arisawa *et al.*, 2002).

Monoclonal antibodies

An alternative approach to the therapy of ATLL is to target cell differentiation markers on the malignant cells with monoclonal antibodies. The high expression of the IL-2 α receptor, CD25, on ATLL cells has made this an attractive target. Waldmann first treated nine patients with ATLL with an anti-CD25 (anti-Tac) monoclonal antibody in the late 1980s. Responses lasting up to 8 months, including one CR, was seen in three patients (Waldmann *et al.*, 1988). Further evaluation of this agent in 19 patients revealed two CR and four PR (Waldmann *et al.*, 1993). Yttrium⁹⁰ labelling of the anti-Tac resulted in a small improvement in response with two CR and seven PR in 18 patients thus treated (Waldmann *et al.*, 1995). Eight patients were treatment naïve prior to the study and five had chronic ATLL. A humanized version of anti-CD25 was used in the next study with PR in 3/11 patients (Morris *et al.*, 2001). A study of humanized anti-CD25 antibody therapy supplementing standard CHOP chemotherapy is currently recruiting patients in the UK.

Another target is CD52. A humanized monoclonal anti-CD52 antibody, Campath-1H, effectively treated SCID mice infected with a tumor-causing HTLV-I-infected cell line. Treated mice surviving as long as HTLV-I unexposed mice (Zhang *et al.*, 2003). A National Institutes of Health (USA) sponsored phase II study of the safety and efficacy of Campath-1H in humans with ATLL (Protocol 03-C-0194) is recruiting (accessed 04/04/2005) http://clinicalstudies.info.nih.gov/detail/A_2003-C-0194.html. Unpublished data from five patients with relapsing ATLL treated at Kumamoto University Hospital indicate that while Campath-1H decreases ATL cells in the peripheral blood, it was not effective against lymphoma. The phenomenon of tumor enlargement during therapy with Campath-1H, previously observed in patients with NHL was also seen (Masao Matsuoka, unpublished).

Transplantation

The first published 'cure' of ATLL following bone marrow transplantation was in 1996. Following a 4-day infusion of cyclophosphamide, etoposide, and doxorubicin, the patient was grafted with cells donated from an HTLV-I-uninfected sister. After 2 years, HTLV-I could not be detected in peripheral blood by nested DNA PCR (Borg *et al.*, 1996) and the patient remains alive, disease free in 2005 (E Tholouli and J Yin, personal communication). Other case reports of allogeneic bone marrow transplantation with CR lasting at least 2 years with or without detectable HTLV-I genome followed (Tajima *et al.*, 2000; Ogata *et al.*, 2002). Molecular remission following autologous stem cell transplantation was reported, but the patient died of an opportunistic infection after 4 months (Nakane *et al.*, 1999). In a case series of 10 patients transplanted with

allogeneic hematopoietic stem cells (Allo-SCT) (9/10 from HLA-identical siblings) after receiving total body irradiation and other conditioning agents, the median leukemia-free survival was >17.5 months but four patients died and in two ATLL relapsed (Utsunomiya *et al.*, 2001). In another series of Allo-SCT, one patient died within 30 days of transplantation but CR was seen in the remaining 10. However, six died of transplantation complications and the overall 1 year survival was only 53% (Kami *et al.*, 2003). The overall median survival from first presentation was >17.3 months and from Allo-SCT >12 months with four patients alive at the time of data census. A recent review of the outcome of 40 patients with acute or lymphoma ATLL, at seven centers in Japan, reported CR in all evaluable cases after Allo-SCT but a median survival time for all patients of only 9.6 months. The estimated 3-year overall survival of 45.3% compares favorably with historical data on chemotherapy. However, comparison across studies is always dangerous given the differences in support over time and potential selection bias (Fukushima *et al.*, 2005). The observation that some ATLL relapses could be successfully managed with a reduction in immune suppression supports the role of the graft versus leukemia effect (Harashima *et al.*, 2004; Okamura *et al.*, 2005).

PUVA can be useful for the management of cutaneous ATLL avoiding the toxicity of chemotherapy and other treatment modalities.

Prevention of ATLL

The routes of transmission of HTLV-I are well documented. ATLL seems to be associated with transmission in early life. Possible factors for this association are the prolonged incubation period between infection and disease and primary infection before maturation of the immune system. Avoidance of breast-feeding by mothers known to carry HTLV-I reduces transmission by 80%. Whether limited breast-feeding for up to 3 months should be allowed, as some data suggest, is a difficult judgment but likely to be influenced by social and cultural factors. Prevention of mother-to-child transmission through breast-feeding should reduce the incidence of ATLL, although this will not be seen for many decades. Whether, HTLV-I infection acquired *in utero* or during delivery carries the same risk of ATLL (as breast milk associated transmission) is unknown. There have been no studies of either antiretroviral therapy or mode of delivery to address the potential for further reducing mother-to-child transmission. Screening for HTLV-I in pregnancy has been introduced in some endemic areas, including Japan and Martinique, but is much less widespread than screening of blood donors. The development of ATLL following blood transfusion acquisition of HTLV-I has rarely been reported.

Alternative approaches to the prevention of ATLL might include reducing HTLV-I proviral load by

antiretroviral therapy, targeted chemotherapy or immunotherapy, whether passive (monoclonal antibodies) or active. One suggestion has been the vaccination of HTLV-I carriers with high proviral load and low HTLV-specific T-cell responses with a HTLV-I Tax-targeted vaccine (Kannagi *et al.*, 2004). ATLL-like lymphoproliferative disease in rats has been prevented by adoptive transfer of T cells immunized with HTLV-I Tax DNA (Ohashi *et al.*, 2000). This is analogous to the control of post-transplantation EBV-associated lymphoproliferation by reducing immune suppression or by infusion of EBV-specific CTL. However, the association

between immune suppression and ATLL is complex. A strong and persistent response to HTLV-I infection is found in patients with inflammatory disease and asymptomatic carriers and to date no association between a lack of response and subsequent development of ATLL has been shown. The proliferation of the malignant cell, diluting, or replacing circulating virus-specific T cells may contribute to any apparent lack of cellular response to HTLV in patients with ATLL. HTLV-I-specific responses can be found in PBL from patients with ATLL (Arnulf *et al.*, 2004).

References

- Ando Y, Ekuni Y, Matsumoto Y, Nakano S, Saito K, Kakimoto K, Tanigawa T, Kawa M and Toyama T. (2004). *J. Obstet. Gynaecol. Res.*, **30**, 436–438.
- Arai F, Miyamoto T, Ohneda O, Inada T, Sudo T, Brasel K, Miyata T, Anderson DM and Suda T. (1999). *J. Exp. Med.*, **190**, 1741–1754.
- Arima N, Mizoguchi H, Shirakawa S, Tomonaga M, Takatsuki K and Ohno R. (1999). *Gan To Kagaku Ryoho*, **26**, 619–629.
- Arisawa K, Katamine S, Kamihira S, Kurokawa K, Sawada T, Soda M, Doi H, Saito H and Shirahama S. (2002). *Cancer Causes Control*, **13**, 657–663.
- Arnulf B, Thorel M, Poirot Y, Tamouza R, Boulanger E, Jaccard A, Oksenhendler E, Hermine O and Pique C. (2004). *Leukemia*, **18**, 126–132.
- Bangham CR. (2003). *Int. J. Hematol.*, **78**, 297–303.
- Barnard AL, Igakura T, Tanaka Y, Taylor GP and Bangham CR. (2005). *Blood*, 14 April, [E-pub ahead of print].
- Besson C, Panelatti G, Delaunay C, Gonin C, Brebion A, Hermine O and Plumelle Y. (2002). *Leuk. Lymphoma*, **43**, 2275–2279.
- Blaheta RA and Cinatl Jr J. (2002). *Med. Res. Rev.*, **22**, 492–511.
- Borg A, Yin JA, Johnson PR, Tosswill J, Saunders M and Morris D. (1996). *Br. J. Haematol.*, **94**, 713–715.
- Cavrois M, Leclercq I, Gout O, Gessain A, Wain-Hobson S and Wattel E. (1998). *Oncogene*, **17**, 77–82.
- Dierov J, Sawaya BE, Prosniak M and Gartenhaus RB. (1999). *Clin. Cancer Res.*, **5**, 2540–2547.
- Doi K, Wu X, Taniguchi Y, Yasunaga JI, Satou Y, Okayama A, Nosaka K and Matsuoka M. (2005). *Blood*, 19 April, [E-pub ahead of print].
- Etoh K, Tamiya S, Yamaguchi K, Okayama A, Tsubouchi H, Ideta T, Mueller N, Takatsuki K and Matsuoka M. (1997). *Cancer Res.*, **57**, 4862–4867.
- Etoh K, Yamaguchi K, Tokudome S, Watanabe T, Okayama A, Stuver S, Mueller N, Takatsuki K and Matsuoka M. (1999). *Int. J. Cancer*, **81**, 859–864.
- Ezaki K, Hirano M, Ohno R, Yamada K, Naito K, Hirota Y, Shirakawa S and Kimura K. (1991). *Cancer*, **68**, 695–698.
- Fontenot JD and Rudensky AY. (2005). *Nat. Immunol.*, **6**, 331–337.
- Franchini G, Fukumoto R and Fullen JR. (2003). *Int. J. Hematol.*, **78**, 280–296.
- Fukushima T, Miyazaki Y, Honda S, Kawano F, Moriuchi Y, Masuda M, Tanosaki R, Utsunomiya A, Uike N, Yoshida S, Okamura J and Tomonaga M. (2005). *Leukemia*, **19**, 829–834.
- Furukawa Y, Kubota R, Tara M, Izumo S and Osame M. (2001). *Blood*, **97**, 987–993.
- Gatza ML, Watt JC and Marriott SJ. (2003). *Oncogene*, **22**, 5141–5149.
- Gill PS, Harrington Jr W, Kaplan MH, Ribeiro RC, Bennett JM, Liebman HA, Bernstein-Singer M, Espina BM, Cabral L, Allen S, Kornblau S, Pike MC and Levine AM. (1995). *N. Engl. J. Med.*, **332**, 1744–1748.
- Han Y, Lassen K, Monie D, Sedaghat AR, Shimoji S, Liu X, Pierson TC, Margolick JB, Siliciano RF and Siliciano JD. (2004). *J. Virol.*, **78**, 6122–6133.
- Harasawa H, Yamada Y, Kudoh M, Sugahara K, Soda H, Hirakata Y, Sasaki H, Ikeda S, Matsuo T, Tomonaga M, Nobori T and Kamihira S. (2002). *Leukemia*, **16**, 1799–1807.
- Harashima N, Kurihara K, Utsunomiya A, Tanosaki R, Hanabuchi S, Masuda M, Ohashi T, Fukui F, Hasegawa A, Masuda T, Takaue Y, Okamura J and Kannagi M. (2004). *Cancer Res.*, **64**, 391–399.
- Hermine O, Allard I, Levy V, Arnulf B, Gessain A and Bazarbachi A. (2002). *Hematol. J.*, **3**, 276–282.
- Hermine O, Bouscary D, Gessain A, Turlure P, Leblond V, Franck N, Buzyn-Veil A, Rio B, Macintyre E, Dreyfus F and Bazarbachi A. (1995). *N. Engl. J. Med.*, **332**, 1749–1751.
- Hermine O, Dombret H, Poupon J, Arnulf B, Lefrere F, Rousselot P, Damaj G, Delarue R, Femand JP, Brouet JC, Degos L, Varet B, de The H and Bazarbachi A. (2004). *Hematol. J.*, **5**, 130–134.
- Igakura T, Stinchcombe JC, Goon PK, Taylor GP, Weber JN, Griffiths GM, Tanaka Y, Osame M and Bangham CR. (2003). *Science*, **299**, 1713–1716.
- Jeang KT, Giam CZ, Majone F and Aboud M. (2004). *J. Biol. Chem.*, **279**, 31991–31994.
- Jones KS, Akel S, Petrow-Sadowski C, Huang Y, Bertolette DC and Ruscetti FW. (2005). *J. Immunol.*, **174**, 4262–4270.
- Jordan A, Bisgrove D and Verdin E. (2003). *EMBO J.*, **22**, 1868–1877.
- Kami M, Hamaki T, Miyakoshi S, Murashige N, Kanda Y, Tanosaki R, Takaue Y, Taniguchi S, Hirai H, Ozawa K and Kasai M. (2003). *Br. J. Haematol.*, **120**, 304–309.
- Kannagi M, Harashima N, Kurihara K, Utsunomiya A, Tanosaki R and Masuda M. (2004). *Expert Rev. Anticancer Ther.*, **4**, 369–376.
- Karube K, Ohshima K, Tsuchiya T, Yamaguchi T, Kawano R, Suzumiya J, Utsunomiya A, Harada M and Kikuchi M. (2004). *Br. J. Haematol.*, **126**, 81–84.
- Katsuki T, Katsuki K, Imai J and Hinuma Y. (1987). *Jpn. J. Cancer Res.*, **78**, 639–642.
- Kiyokawa T, Yamaguchi K, Takeya M, Takahashi K, Watanabe T, Matsumoto T, Lee SY and Takatsuki K. (1987). *Cancer*, **59**, 1187–1191.

- Koiwa T, Hamano-Usami A, Ishida T, Okayama A, Yamaguchi K, Kamihira S and Watanabe T. (2002). *J. Virol.*, **76**, 9389–9397.
- Krueger A, Fas SC, Baumann S and Krammer PH. (2003). *Immunol. Rev.*, **193**, 58–69.
- Kuramochi M, Fukuhara H, Nobukuni T, Kanbe T, Maruyama T, Ghosh HP, Pletcher M, Isomura M, Onizuka M, Kitamura T, Sekiya T, Reeves RH and Murakami Y. (2001). *Nat. Genet.*, **27**, 427–430.
- Kuwazuru Y, Hanada S, Furukawa T, Yoshimura A, Sumizawa T, Utsunomiya A, Ishibashi K, Saito T, Uozumi K, Maruyama M, Ishizawa M, Arima T and Akiyama S-I. (1990). *Blood*, **76**, 2065–2071.
- Li HC, Biggar RJ, Miley WJ, Maloney EM, Cranston B, Hanchard B and Hisada M. (2004). *J. Infect. Dis.*, **190**, 1275–1278.
- Lymphoma Study Group Shimoyama M, Ota K, Kikuchi M, Tominaga S and Yunoki K. (1982). *Jpn J Clin Oncol*, **12**, 227–238.
- Mahieux R and Hermine O. (2005). *Leuk Lymphoma*, **46**, 347–355.
- Makino T, Nakahara K, Takatsuka Y, Shimotakahara S, Utsunomiya A, Hanada S, Tokunaga M and Arima T. (1994). *Rinsho Ketsueki*, **35**, 42–48.
- Manel N, Kim FJ, Kinet S, Taylor N, Sitbon M and Battini JL. (2003). *Cell*, **115**, 449–459.
- Matsuoka M and Jeang KT. (2005). *Cancer Res.*, **65**, 4467–4470.
- Matsushita K, Matsumoto T, Ohtsubo H, Fujiwara H, Imamura N, Hidaka S, Kukita T, Tei C, Matsumoto M and Arima N. (1999). *Leuk. Lymphoma*, **36**, 67–75.
- Matutes E, Taylor GP, Cavenagh J, Pagliuca A, Bareford D, Domingo A, Hamblin M, Kelsey S, Mir N and Reilly JT. (2001). *Br. J. Haematol.*, **113**, 779–784.
- Mercieca J, Matutes E, Dearden C, MacLennan K and Catovsky D. (1994). *J. Clin. Oncol.*, **12**, 2588–2593.
- Mittelstadt PR and Ashwell JD. (1998). *Mol. Cell. Biol.*, **18**, 3744–3751.
- Mori N, Matsuda T, Tadano M, Kinjo T, Yamada Y, Tsukasaki K, Ikeda S, Yamasaki Y, Tanaka Y, Ohta T, Iwamasa T, Tomonaga M and Yamamoto N. (2004). *J. Virol.*, **78**, 4582–4590.
- Mori N, Yamada Y, Ikeda S, Yamasaki Y, Tsukasaki K, Tanaka Y, Tomonaga M, Yamamoto N and Fujii M. (2002). *Blood*, **100**, 1828–1834.
- Morris JC, Janik JE, Turner M, Lee C, Top L, Jaffe ES, Stetler-Stevenson M, Blessing JJ, Fleisher TA and Waldmann T. (2001). *10th International Conference on Human Retrovirology: HTLV and Related Viruses, Vol. 17 Suppl. AIDS Res Hum Retroviruses: Dublin*, pp. S29.
- Nakane M, Ohashi K, Sato Y, Moriya A, Inoue T, Mori S, Tanikawa S, Akiyama H, Maeda Y, Sasaki T, Karasawa K, Okuyama Y, Hiruma K and Sakamaki H. (1999). *Bone Marrow Transplant*, **24**, 219–221.
- Nasr R, Rosenwald A, El-Sabban ME, Arnulf B, Zalloua P, Lepelletier Y, Bex F, Hermine O, Staudt L, de The H and Bazarbachi A. (2003). *Blood*, **101**, 4576–4582.
- Nosaka K, Miyamoto T, Sakai T, Mitsuya H, Suda T and Matsuoka M. (2002). *Blood*, **99**, 634–640.
- Ogata M, Ogata Y, Imamura T, Ohtsuka E, Kikuchi H, Utsunomiya A, Yashiki S, Sonoda S and Nasu M. (2002). *Bone Marrow Transplant*, **30**, 699–701.
- Ohashi T, Hanabuchi S, Kato H, Tateno H, Takemura F, Tsukahara T, Koya Y, Hasegawa A, Masuda T and Kannagi M. (2000). *J. Virol.*, **74**, 9610–9616.
- Ohno R, Masaoka T, Shirakawa S, Sakamoto S, Hirano M, Hanada S, Yasunaga K, Yokomaku S, Mitomo Y, Nagai K, Yamada K and Furue H. (1993). *Cancer*, **71**, 2217–2221.
- Okamura J, Utsunomiya A, Tanosaki R, Uike N, Sonoda S, Kannagi M, Tomonaga M, Harada M, Kimura N, Masuda M, Kawano F, Yufu Y, Hattori H, Kikuchi H and Saburi Y. (2005). *Blood*, **105**, 4143–4145.
- Okayama A, Stuver S, Matsuoka M, Ishizaki J, Tanaka G, Kubuki Y, Mueller N, Hsieh CC, Tachibana N and Tsubouchi H. (2004). *Int. J. Cancer*, **110**, 621–625.
- Okochi K, Sato H and Hinuma Y. (1984). *Vox. Sang*, **46**, 245–253.
- Pawson R, Richardson DS, Pagliuca A, Kelsey SM, Hoque S, Breuer J, Newland AC and Mufti GJ. (1998). *Leuk. Lymphoma*, **31**, 177–185.
- Sasaki H, Nishikata I, Shiraga T, Akamatsu E, Fukami T, Hidaka T, Kubuki Y, Okayama A, Hamada K, Okabe H, Murakami Y, Tsubouchi H and Morishita K. (2005). *Blood*, **105**, 1204–1213.
- Satou Y, Nosaka K, Koya Y, Yasunaga JI, Toyokuni S and Matsuoka M. (2004). *Leukemia*, **18**, 1357–1363.
- Shimoyama M. (1992). *Advances in Adult T-cell Leukaemia and HTLV-I Research*. Takatsuki K, Hinuma Y, Yoshida M (eds). Japanese Scientific Societies Press: Tokyo, pp. 43–56.
- Shimoyama M, Ota K, Kikuchi M, Yunoki K, Konda S, Takatsuki K, Ichimaru M, Tominaga S, Tsugane S and Minato K. (1988). *J. Clin. Oncol.*, **6**, 1088–1097.
- Siegel RS, Gartenhaus RB and Kuzel TM. (2001). *Curr. Treat. Options Oncol.*, **2**, 291–300.
- Taguchi H, Kinoshita KI, Takatsuki K, Tomonaga M, Araki K, Arima N, Ikeda S, Uozumi K, Kohno H, Kawano F, Kikuchi H, Takahashi H, Tamura K, Chiyoda S, Tsuda H, Nishimura H, Hosokawa T, Matsuzaki H, Momita S, Yamada O and Miyoshi I. (1996). *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.*, **12**, 182–186.
- Taguchi T, Ohta K, Hotta T, Shirakawa S, Masaoka T and Kimura I. (1997). *Gan To Kagaku Ryoho*, **24**, 1263–1271.
- Tajima K, Amakawa R, Uehira K, Matsumoto N, Shimizu T, Miyazaki Y, Fujimoto M, Kishimoto Y and Fukuhara S. (2000). *Int. J. Hematol.*, **71**, 290–293.
- Takeda S, Maeda M, Morikawa S, Taniguchi Y, Yasunaga J, Nosaka K, Tanaka Y and Matsuoka M. (2004). *Int. J. Cancer*, **109**, 559–567.
- Tamiya S, Matsuoka M, Etoh K, Watanabe T, Kamihira S, Yamaguchi K and Takatsuki K. (1996). *Blood*, **88**, 3065–3073.
- Tamiya S, Matsuoka M, Takemoto S, Shimizu K, Yamaguchi K, Mita S, Uchino M, Ando M, Miyaguni T, Kuniyoshi K and Takatsuki K. (1995). *Leukemia*, **9**, 1768–1770.
- Tan C and Waldmann TA. (2002). *Cancer Res.*, **62**, 1083–1086.
- Tanaka G, Okayama A, Watanabe T, Aizawa S, Stuver S, Mueller N, Hsieh CC and Tsubouchi H. (2005). *J. Infect. Dis.*, **191**, 1140–1147.
- Taylor GP, Gessain A, Popescu DM, Zehender G, Padua E, Soriano V, Anderson S, Tosswill JHC and Weber JN. (2001). *AIDS Res. Hum. Retroviruses*, **17** (Suppl 1), S-69.
- Thompson C and Powrie F. (2004). *Curr. Opin. Pharmacol.*, **4**, 408–414.
- Tobinai K, Shimoyama M, Inoue S, Takayasu S, Mikuni C, Kozuru M, Oda S and Nakajima H. (1992). *Jpn. J. Clin. Oncol.*, **22**, 164–171.
- Tobinai K, Shimoyama M, Takatsuki K, Tomonaga M, Araki K and Arima N. (1994). *Proc. Am. Soc. Oncol.*, **13**, 378.
- Tobinai K, Uike N, Saburi Y, Chou T, Etoh T, Masuda M, Kawano F, Matsuoka M, Taguchi H, Makino T, Asano Y,

- Tamura K and Ohashi Y. (2003). *Int. J. Hematol.*, **77**, 512–517.
- Toyota M, Ho C, Ahuja N, Jair KW, Li Q, Ohe-Toyota M, Baylin SB and Issa JP. (1999). *Cancer Res.*, **59**, 2307–2312.
- Tsuda H, Takatsuki K, Ohno R, Masaoka T, Okada K, Shirakawa S, Ohashi Y and Ota K. (1994). *Br. J. Cancer*, **70**, 771–774.
- Tsukasaki K, Ikeda S, Murata K, Maeda T, Atogami S, Sohda H, Momita S, Jubashi T, Yamada Y, Mine M, Kamihira S and Tomonaga M. (1993). *Leuk. Res.*, **17**, 157–166.
- Tsukasaki K, Tobinai K, Shimoyama M, Kozuru M, Uike N, Yamada Y, Tomonaga M, Araki K, Kasai M, Takatsuki K, Tara M, Mikuni C and Hotta T. (2003). *Int. J. Hematol.*, **77**, 164–170.
- Uike N, Choi I, Tokoro A, Goto T, Yufu Y, Kozuru M and Tobinai K. (1998). *Intern. Med.*, **37**, 411–413.
- Uozumi K, Hanada S, Ohno N, Ishitsuka K, Shimotakahara S, Otsuka M, Chyuman Y, Nakahara K, Takeshita T, Kuwazuru Y, Saito T, Makino T, Iwahashi M, Utsunomiya A and Arima T. (1995). *Leuk. Lymphoma*, **18**, 317–323.
- Utsunomiya A, Miyazaki Y, Takatsuka Y, Hanada S, Uozumi K, Yashiki S, Tara M, Kawano F, Saburi Y, Kikuchi H, Hara M, Sao H, Morishima Y, Kadera Y, Sonoda S and Tomonaga M. (2001). *Bone Marrow Transplant*, **27**, 15–20.
- Waldmann TA, Goldman CK, Bongiovanni KF, Sharrow SO, Davey MP, Cease KB, Greenberg SJ and Longo DL. (1988). *Blood*, **72**, 1805–1816.
- Waldmann TA, White JD, Carrasquillo JA, Reynolds JC, Paik CH, Gansow OA, Brechbiel MW, Jaffe ES, Fleisher TA, Goldman CK, Top LE, Bamford R, Zaknoen E, Roessler E, Kasten-Sportes C, England R, Litou H, Johnson JA, Jackson-White T, Manns A, Hanchard B, Junghans RP and Nelson DL. (1995). *Blood*, **86**, 4063–4075.
- Waldmann TA, White JD, Goldman CK, Top L, Grant A, Bamford R, Roessler E, Horak ID, Zaknoen S, Kasten-Sportes C, England R, Horak E, Mishra B, Dipre M, Hale P, Fleisher TA, Junghans RP, Jaffe ES and Nelson DL. (1993). *Blood*, **82**, 1701–1712.
- Watanabe T, Yamaguchi K, Takatsuki K, Osame M and Yoshida M. (1990). *J. Exp. Med.*, **172**, 759–765.
- Welles SL, Tachibana N, Okayama A, Shioiri S, Ishihara S, Murai K and Mueller NE. (1994). *Int. J. Cancer*, **56**, 337–340.
- Wu X, Li Y, Crise B and Burgess SM. (2003). *Science*, **300**, 1749–1751.
- Yamada Y, Hata Y, Murata K, Sugawara K, Ikeda S, Mine M, Maeda T, Hirakata Y, Kamihira S, Tsukasaki K, Ogawa S, Hirai H, Koefler HP and Tomonaga M. (1997). *J. Clin. Oncol.*, **15**, 1778–1785.
- Yamada Y and Tomonaga M. (2003). *Leuk. Lymphoma*, **44**, 611–618.
- 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 and Shimoyama M. (2001). *Br. J. Haematol.*, **113**, 375–382.
- Yasunaga J, Sakai T, Nosaka K, Etoh K, Tamiya S, Koga S, Mita S, Uchino M, Mitsuya H and Matsuoka M. (2001). *Blood*, **97**, 3177–3183.
- Yasunaga J, Taniguchi Y, Nosaka K, Yoshida M, Satou Y, Sakai T, Mitsuya H and Matsuoka M. (2004). *Cancer Res.*, **64**, 6002–6009.
- Yoshida M. (2001). *Annu. Rev. Immunol.*, **19**, 475–496.
- Yoshida M, Nosaka K, Yasunaga J, Nishikata I, Morishita K and Matsuoka M. (2004). *Blood*, **103**, 2753–2760.
- Zhang Z, Zhang M, Goldman CK, Ravetch JV and Waldmann TA. (2003). *Cancer Res.*, **63**, 6453–6457.