Adult T-cell leukemia (ATL) is a highly chemoresistant and usually fatal T-cell malignancy due to the human T-cell lymphotropic virus-1 (HTLV-1). About 3% to 5% of them develop adult T-cell leukemia (ATL), an aggressive malignancy of CD4⁺CD25⁺ T lymphocytes with profound public health impact in HTLV-1 endemic areas. Complete responses even with intensive chemotherapy are rare, and survival is only 6 to 24 months. A dysfunctional TP53 pathway and constitutive nuclear factor-κB (NF-κB) activation contribute to chemoresistance. Moreover, the definition and monitoring of subclinical disease is challenging. While HTLV-1 viral loads increase with progression from smoldering to acute phase and with increasing tumor burden, changes following therapy in ATL have not been well characterized. Alemtuzumab is a humanized immunoglobulin G1 κ (IgG1 κ) monoclonal antibody directed against CD52, a glycosyl-phosphatidylinositol (GPI)–linked protein expressed on normal and malignant leukocytes including ATL cells. Alemtuzumab is active in a mouse model of ATL.

Study design
Approval for this study was obtained from the OSU Institutional Review Board (OSU 1997CO194). Informed consent was provided according to the Declaration of Helsinki. Quantitative real-time polymerase chain reaction (PCR) was performed with genomic DNA from peripheral blood mononuclear cells (PBMCs) using established primers and conditions. As control, 18S rRNA gene copies were measured simultaneously in separate tubes using an 18S standard curve. Triplicate sample, standard curves, and no-template control reactions were performed using the Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). Data were analyzed using SDS v1.9 software. Average PCR efficiencies of the tax and r18S standard curves were 103% ± 3.7% and 99.9% ± 3.0%, respectively. Interassay and intra-assay variabilities and sensitivities obtained were similar to those described previously. Copy numbers of the tax gene were first normalized to 18S copies and then to 1 μg total DNA. For cytotoxicity, fresh ATL cells from subject “ATL-1” and archived primary acute ATL cells from another patient (ATL-2), both procured with IRB approval, were incubated for 4 hours in media with alemtuzumab and anti-Fc IgG (both 10 mg/mL), alemtuzumab (10 mg/mL) with PBMCs at an effector-target ratio of 25:1, or alemtuzumab (10 mg/mL) in media with 30% human serum. Cell death was determined by propidium iodide (PI) staining and fluorescence-activated cell sorter (FACS) analysis for apoptosis and complement-dependent cytotoxicity (CDC) or by chromium-51 release assay for antibody-dependent cellular cytotoxicity (ADCC). Genomic DNA from ATL-1 and ATL-2 cells was amplified using primers for TP53 exons 5 to 9 and assessed for mutation using denaturing gradient gel electrophoresis and confirmatory sequencing of mutations as previously published.

Results and discussion
A 63-year-old woman was diagnosed with chronic ATL in July 2000. Oral chlorambucil and cyclophosphamide produced a modest reduction of the lymphocytosis, but in July 2002 she developed peripheral blood (PB) and biopsy-proven cutaneous progression. Interferon-α (IFN-α) was started in combination with zidovudine (AZT), stavudine (d4T), and lamivudine (3TC). However, after variable intervals, antiviral drugs were discontinued due to toxicity. Durable hematologic complete response and suppression of HTLV-1 viral load following alemtuzumab in zidovudine/IFN-α-refractory adult T-cell leukemia

Andrew Mone, Shannon Puhalla, Susan Whitman, Robert A. Baiocchi, Julio Cruz, Tamara Vukosavljevic, Amy Banks, Charles F. Eisenbeis, John C. Byrd, Michael A. Caligiuri, and Pierluigi Porcu
In January 2003, thalidomide was added to IFN-α. Both drugs were stopped in March 2003, when the patient presented to The Ohio State University with CD4+ T-cell lymphocytosis (6.4 × 10^9/L) and new deforming cutaneous lesions. HTLV-1 serology and PCR on PBMCs were positive. Computed tomography (CT) scans showed no lymphadenopathy, hepatosplenomegaly, or pulmonary or bone lesions. Serum calcium levels were normal. Serum lactate dehydrogenase (LDH) was 265 IU/mL (normal: 190 IU/mL or below). The bone marrow was extensively involved by an abnormal population of T cells expressing CD2, CD3, CD4, CD5, CD25, and CD52 but negative for CD7, CD8, and CD26. Denileukin difitox, 18 μg/kg/d for 5 days every 3 weeks, resulted in near disappearance of the cutaneous lesions but only modest improvement of the lymphocytosis (Figure 1), and after 10 cycles (October 2003), was discontinued. By November 2003, progression was already noted and reinitiation of denileukin difitox was ineffective. Alemtuzumab (30 mg subcutaneously thrice weekly for 12 weeks) was started. Within 4 weeks, skin and PB had cleared (Figure 1). A bone marrow biopsy at completion of alemtuzumab (May 2004) and 3 months later (December 2004) showed no morphologic or immunophenotypic evidence of disease. All therapy was outpatient. Neutropenia and asymptomatic cytomegalovirus (CMV) reactivation developed after 8 to 9 weeks of therapy. Alemtuzumab was held for 2 weeks, the neutrophil count recovered on filgrastim, and CMV was rapidly cleared with valganciclovir. At completion of alemtuzumab persistent neutropenia required intermittent use of filgrastim. As of May 2005, the patient had no clinical or hematologic evidence of disease, normal neutrophil count off filgrastim, and normal lymphocyte count.

While denileukin difitox only resulted in a cutaneous response, alemtuzumab produced a dramatic decline of PB lymphocytosis and HTLV-1 viral load, which remained at the lower limit of detection, even at recovery of PB CD4+ T cells after alemtuzumab (Figure 1). Analysis of cytotoxicity with the patient’s cells (ATL-1) and with an archived sample of primary acute ATL cells (ATL-2) showed that, in the presence of human serum, alemtuzumab effectively induced CDC (65% cytotoxicity at 4 hours), whereas ADCC and direct apoptosis were negligible (Figure 2). The cytotoxicity of alemtuzumab in ATL-2 cells carrying a nonsense exon 5 TP53 mutation (data not shown) parallels that observed in CLL with 17q deletion and/or mutations.12

The activity of alemtuzumab in a mouse model of ATL3 and in patients with other refractory T-cell malignancies or CLL with mutated or absent TP5312,13–16 provides a strong rationale for its use in ATL. The clinical response and durable HTLV-1 viral load reduction achieved in this patient are very encouraging because comparable reductions in tumor burden and viral loads have only been observed with allogeneic hematopoietic stem cell transplantation (HSCT).17–19 Although its use in ATL has been previously cited20 and, more recently, early phase 2 results with single-agent intravenous alemtuzumab were presented in abstract form,21 this is the first analysis of the combined effects of subcutaneous alemtuzumab on clinical response, HTLV-1 viral loads, and in vitro cytotoxicity in ATL. The relevant mechanism of response to alemtuzumab in ATL remains undefined. While the activity of alemtuzumab in the model of Zhang et al22 requires activating Fcγ receptors, CDC was the dominant in vitro mechanism in our 2 ATL samples. In CLL high-affinity alleles for FcγRIIIa and FcγRIIIb do not correlate with clinical response to alemtuzumab in vivo, suggesting FcγR-independent mechanisms.22,23 Studies of FcγR polymorphism and ADCC in T-cell lymphoma patients treated with alemtuzumab are in progress as part of a prospective study.

The observed normalization of CD4+ T cells after alemtuzumab despite persistently suppressed HTLV-1 viral loads is important because of appropriate concerns about the immunosuppressive properties of alemtuzumab. In principle, alemtuzumab could lead not only to opportunistic infections, but also to disease acceleration in ATL by further impairing immune surveillance. However, immune responses in ATL after therapy have not been well studied outside of allogeneic HSCT, where hematopoietic reconstitution is due to donor-derived HTLV-1–negative cells.24 Recovery of autologous CD4+ T cells without a rise in HTLV-1 viral load, as seen here, could reflect the expansion of endogenous HTLV-1–negative T cells, suggesting that alemtuzumab may have some selectivity for HTLV-1–infected T cells. This hypothesis is consistent with the current theory that expansion of HTLV-1–infected T cells is the predominant mechanism of maintenance of proviral load.24 The role of pre-exposure to denileukin difitox in this patient’s response to alemtuzumab could not be addressed, but clinical trials with alemtuzumab in combination with immunotoxins, chemotherapy, or antivirals should be considered. The role of maintenance alemtuzumab vis-à-vis HTLV-1 viral loads should also be studied. The safety and ease of administration of subcutaneous alemtuzumab in CLL23 suggest that this may be a feasible therapy even in areas remote from tertiary care centers, where most ATL patients live.

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References


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