Effective treatment of a murine model of adult T-cell leukemia using Depsipeptide and its combination with unmodified daclizumab directed toward CD25

Jing Chen¹, Meili Zhang¹, Wei Ju¹ and Thomas A. Waldmann¹

¹Metabolism Branch, Center for Cancer Research, National Cancer Institute, 10 Center Drive, Bethesda, MD 20892-1374

Running Title: Therapy of ATL with depsipeptide and Daclizumab

Category: Neoplasia

Correspondence should be addressed to:

Dr. Thomas A. Waldmann
Building 10, Room 4N115
10 Center Drive, National Institutes of Health
Bethesda, MD 20892-1374
Phone: (301) 496-6656
Fax: (301) 496-9956
E-mail: tawald@helix.nih.gov
Abstract

Adult T-cell leukemia (ATL) is caused by human T-cell lymphotropic virus I (HTLV-1) and is an aggressive malignancy of CD4, CD25 expressing leukemia and lymphoma cells. There is no accepted curative therapy for ATL. Depsipeptide, a histone deacetylase inhibitor, has demonstrated major anti-tumor effects in leukemias and lymphomas. In this study, we investigated the therapeutic efficacy of depsipeptide alone and in combination with daclizumab (HAT, humanized anti-Tac) in a murine model of human ATL. The Met-1 ATL model was established by intraperitoneal injection of ex vivo leukemic cells into NOD/SCID mice. Either depsipeptide, given at 0.5mg/kg every other day for 2 weeks, or daclizumab, given at 100ug weekly for 4 weeks, inhibited tumor growth as monitored by serum levels of soluble IL-2Rα (sIL-2Rα) and soluble β2-microglobulin(β2µ) (P<0.0001), and prolonged survival of the leukemia-bearing mice (P<0.0001) as compared with the control group. Combination of depsipeptide with daclizumab enhanced the anti-tumor effect, as shown by both sIL-2Rα and β2µ levels and survival of the leukemia-bearing mice, when compared with those in the depsipeptide or daclizumab alone groups (P<0.001). The significantly improved therapeutic efficacy by combining depsipeptide with daclizumab supports a clinical trial of this combination in the treatment of ATL.
**Introduction**

Adult T-cell leukemia (ATL) is an aggressive malignancy of mature activated CD4+ T-cells associated with human T-cell lymphotrophic virus type 1 (HTLV-1) infection. The leukemic cells are characterized by the expression of interleukin-2 receptor α (IL-2Ra, CD25) on their cell surfaces. At present, there is no accepted curative therapy for ATL and the patients progress to death with a median survival duration of 9 months in acute ATL and 24 months in chronic ATL. A preclinical *in vivo* murine model of ATL was developed by introducing leukemic cells (Met-1) from a patient with ATL into nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. New therapeutic approaches have been tested in this model before initiating human clinical trials.

Daclizumab (Zenapax®), a monoclonal antibody targeted at the IL-2Ra (CD25) showed excellent therapeutic efficacy in this murine model. In clinical trials, it has also yielded some partial or rare complete remissions in patients with ATL, suggesting that this new IL-2Ra targeting monoclonal antibody therapy is promising in the treatment of patients with ATL. Recently, a high response rate following azidothymidine/interferon α (AZT/IFN-α) treatment of ATL patients has been reported in several human trials. The tumor suppressor p53 appears to be a predictive marker for AZT response as patients responded to AZT therapy only when p53 was wild type in sequence, and inversely disease relapse or absence of response was associated with mutation and inactive p53. A paradigm has emerged that the combination of a monoclonal antibody with chemotherapeutic reagents that function via different mechanisms of action may be greater than additive in their cytotoxic action leading to malignant cell death. Thus, it will
be of great value to find chemotherapy reagents that could enhance the anti-tumor efficacy of daclizumab.

Histone deacetylase (HDAC) inhibitors are a new class of anti-tumor agents which are currently undergoing intensive pre-clinical and clinical testing. HDAC inhibitors are potent inducers of apoptosis and growth inhibition with a variety of transformed cells in vitro and in vivo, including malignancies originating from lymphoid cells\textsuperscript{15-17}.

Depsipeptide (FR901228, FK228), isolated from \textit{Chronobacterium violaceum}, is a member of the cyclic peptide class of HDAC inhibitors. Depsipeptide has shown cytotoxic effects on a number of malignant lymphoid cell lines\textsuperscript{18} including HTLV-1 infected T-cell lines\textsuperscript{19}. Depsipeptide is currently in clinical trials for evaluation of its anticancer efficacy\textsuperscript{20}. Recent results using FR901228 in patients with cutaneous and peripheral T-cell lymphoma suggest significant activity in these diseases\textsuperscript{15}. Furthermore, the study shows that the IL-2Ra expression level on the patients’ malignant cells after depsipeptide treatment is increased. Based on these findings, it has been speculated that the combination of depsipeptide with IL-2Ra targeting therapy may enhance the anti-tumor efficacy of depsipeptide\textsuperscript{15,21}. As discussed above, daclizumab is an IL-2Ra targeting agent. Thus, the combination of depsipeptide with daclizumab in the treatment of T cell malignancy could enhance the anti-tumor efficacy not only through two different modes of killing, but also through enhancing the expression of the target of daclizumab.

In this study, we investigated the therapeutic efficacy of depsipeptide alone and its combination with daclizumab in a murine model of ATL. Both depsipeptide and
daclizumab inhibited tumor growth as monitored by soluble IL-2Rα and soluble β2µ levels (P<0.0001), and prolonged the survival of the leukemia–bearing mice significantly (P<0.0001). Combination of these two agents demonstrated much greater therapeutic efficacy in the Met-1 model of ATL. The median survival of leukemia-bearing mice was 180 days in the combination group, compared to 113 days in the depsipeptide alone group, 98 days in the daclizumab group and 57 days in the PBS group. Our data would support the use of depsipeptide, preferably, combined with daclizumab, in the treatment of patients with ATL.

**Materials and methods**

**Drug and antibody**

Depsipeptide was obtained from Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute (Bethesda, MD). Daclizumab which recognizes IL-2Rα, was acquired from Hoffmann-La Roche (Nutley, NJ)22.

**Proliferation assay**

HTLV-1 positive T-cell lines, HUT102, CaGT, MT-2, MT-1 and MJ, were maintained in RPMI 1640 containing 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin in an atmosphere containing 5% CO2. Aliquots of 5 x 10^3 cells were seeded in 96-well culture plates and incubated with medium alone or with serial dilutions of depsipeptide (0.125ng/ml, 0.5ng/ml, 2ng/ml and 5ng/ml). The cells were pulsed after 72 hours of culture for 6 hours with 1 µCi (0.037 MBq) [³H]thymidine (Amersham, Piscataway, New Jersey). Then, the cells were harvested with a 96-well
harvester (Tomtec, Hamden, CT) and counted in a counter (Wallac, Turku, Finland).
The assay was performed in triplicate and repeated 3 times.

**Annexin-V staining and apoptosis analysis**

Quantification of apoptosis was performed by immunostaining cells with Annexin-V, which specifically detects phospholipid phosphatidylserine (PS) redistributed from the inner to the outer leaflet during apoptosis. Cells cultured with 2ng/ml depsipeptide or media for 24 and 48 hours were labeled with Annexin V-FITC and subsequently analyzed by flow cytometry (FACS). The dead cells were labeled by propidium iodide (PI).

**Caspase-3 and caspase-9 activity assay**

The caspase-3 and caspase-9 activities were measured using caspase-3 and caspase-9 colorimetric assays from R&D systems (Minneapolis, MN). The activity was expressed as fold increase in depsipeptide treated cells over that of non-treated cells. The background values were subtracted from the experimental results prior to calculating the fold induction.

**Western Blot Analysis**

Treated cells were solubilized at 4°C in RIPA lysis buffer (50mM Tris-CL, pH7.4, 0.5% sodium deoxycholate, 1% Nonidet P-40, 150mM NaCl, 66-µg/ml aprotinin, 100-µg/ml phenylmethylsulfonyl fluoride, and 1 mM sodium orthovanadate). Cell lysates (50 µg) were resolved by electrophoresis on SDS-polyacrylamide (4-12%) gels and transferred to
polyvinylidene difluoride membranes. After blocking of the membranes in 5% skim milk and 0.05% Tween 20 in Tris-buffered saline, the blots were incubated with the mouse monoclonal antibody to p21, cyclin D1, Bcl-2, \( \alpha \)-tubulin (Santa Cruz Biotechnology, Santa Cruz, CA) or the rabbit polyclonal antibody to cyclin A (Santa Cruz), acetyl-histone H3, histone H3 (Cell signaling Technology, Danvers, MA) or Bcl-X\(_L\) (Transduction Laboratories, Lexington, KY). The anti-Tax antibody was kindly provided by Dr. Steven Jacobson (NIND, NIH). After several washes, the protein bands recognized by the antibodies were visualized with an enhanced chemiluminescence Western blotting detection system (Amersham).

**Mouse model of ATL**

The ATL cell population, MET-1, was established from the peripheral blood of a patient with acute ATL and the cells were maintained by serial transfer in NOD/SCID mice (Jackson Laboratories, Bar Harbor, ME). MET-1 cells have a distinct phenotype elucidated by fluorescein-activating cell sorting (FACS) analysis: CD3\(^{dim}\), CD4\(^{+/-}\), CD7\(^-\), CD20\(^-\), and CD25\(^+\). The leukemia model was established by intraperitoneal injection of \(1.5 \times 10^7\) MET-1 cells into NOD/SCID mice as described previously\(^8\). The therapy experiments were performed on these mice when their serum soluble IL-2R\(\alpha\) (sIL-2R\(\alpha\)) levels were more than 1000 pg/mL, which occurs approximately 10 to 14 days after tumor inoculation. All animal experiments were performed in accordance with National Institutes of Health Animal Care and Use Committee guidelines.

**Definition of the maximum tolerated dose**
Prior to the initiation of the therapeutic studies, the maximum tolerated dose of depsipeptide was determined in NOD/SCID mice. Doses of 0.125, 0.25, 0.5, 1.0, and 2.0 mg depsipeptide per kg body weight were administered intraperitoneally daily for two weeks. All mice in 2.0mg/kg group died at day 7 and 80% of the mice in 1.0mg/kg group died at day 14. The mice in the 0.5, 0.25 and 0.125 mg/kg group were still alive six months after depsipeptide injection. Therefore, a dose of 0.5 mg/kg every other day for 14 days was chosen to use in the therapeutic trials and the dose is consistent with what people have used in mice\textsuperscript{19,25}.

**Therapy study**

Therapeutic studies were performed in MET-1 leukemia-bearing mice with serum surrogate tumor marker sIL-2R\(\alpha\) values of 1000 -10,000 pg/mL in the small tumor burden trial and 10,000 – 25,000 pg/ml in the large tumor burden trial. There were 5 groups in the therapeutic trials. Group 1, the depsipeptide group, received intraperitoneal injections of 0.5 mg depsipeptide/kg body weight every other day for two weeks. Group 2, the immunotherapy (daclizumab) group, was given intravenous injections of 100\(\mu\)g daclizumab on days 0, 7, 14, and 21. Group 3, the combination therapy group, received a combined therapy of depsipeptide and daclizumab (dosing schedule as in group 1 plus group 2). Group 4 received 200\(\mu\)L PBS weekly for 4 weeks served as a control. Group 5, with no tumor and no therapy and served as a control for the natural death of NOD/SCID mouse. There were 13 mice per group in the small tumor burden therapeutic trial(sIL-2R\(\alpha\), 1,000 – 10,000 pg/ml); and there were 8 mice per group in the large tumor burden trial (sIL-2R\(\alpha\), 10,000 -25,000pg/ml). The groups were randomly assigned and had
comparable average levels of the surrogate tumor marker, sIL-2Rα, at the beginning of the experiments.

**Monitoring of tumor growth**

Measurements of the serum concentrations of the soluble IL-2Rα or soluble β2-microglobulin (β2µ) were performed using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The ELISAs were performed following the manufacturer's recommendation.

**Statistical analysis**

The serum levels of human sIL-2Rα and β2µ were analyzed at different time points for the different treatment groups using the Student t test for unpaired data. Statistical significance of differences in survival of mice in different groups was determined by the log-rank test using the StatView program (Abacus Concepts, Berkeley, CA).

**Results**

**Depsipeptide inhibited the proliferation and induced apoptosis in HTLV-1 positive T-cell lines.** We first examined the effects of depsipeptide on proliferation and apoptosis of HTLV-1 infected T-cell lines. The cell lines (Hut102, MT-2, CaGT, MT-1 and MJ) were treated with various concentrations (0.12ng/ml to 5ng/ml) of depsipeptide for 72hrs. Depsipeptide inhibited the proliferation in a dose-dependent manner in all five cell lines tested (Fig1.A). Staining of depsipeptide treated cells with Annexin-V FITC showed that a significant proportion of the cells had undergone apoptosis 24 and 48 hours after
depsipeptide treatment (2ng/ml) (Fig.1B). Both caspase-9 and caspase-3 activities were induced in the HTLV-1 infected cell lines 24 hours after depsipeptide treatment (5ng/ml) (Fig.1C). It has been reported by Dr. Nguyen et al. that depsipeptide induced apoptosis of lung or esophageal cancer cells by activating the mitochondria-dependent death-signaling pathway26. Our data suggested a similar action of depsipeptide in HTLV-1 infected cell lines.

**Depsipeptide induced accumulation of histone acetylation and affected the expression of intracellular regulators of cell cycle and apoptosis.** To clarify the molecular mechanisms by which depsipeptide induces inhibition of cell growth and apoptosis in HTLV-1 infected T-cell lines, we examined the histone acetylation level (histone H3) and some regulators of the cell cycle (cyclin A, cyclin D1, p21) and apoptosis (Bcl-2, Bcl-X<sub>L</sub>) by western blot analysis. As expected, the acetylation level of histone H3 was dramatically increased in depsipeptide treated (5ng/ml) HTLV-1 positive Hut102 and MT-2 cells both at 24 and 48 hours after treatment (Fig.2A). The accumulation of histone acetylation levels could profoundly affect the transcription of genes involved in proliferation, cell cycle and apoptosis 27. As shown in Fig. 2B, the expression levels of the CDK inhibitor p21 were up-regulated and the expressions of cyclin A were down-regulated in depsipeptide treated (5ng/ml) Hut102 and MT-2 cells; while the expression of cyclin D1 was not altered by depsipeptide. Furthermore, the expressions of anti-apoptotic proteins Bcl-2 and Bcl-X<sub>L</sub> were decreased in depsipeptide treated Hut102 and MT-2 cells, which is consistent with the observation that depsipeptide induced apoptosis in these cell lines. Interestingly, depsipeptide treatment induced the viral gene Tax expression in Hut102 and MT-2 cells. It has been reported that HDAC1
negatively regulates viral gene expression and the HDAC inhibitor trichostatin A (TSA)
induced viral gene Tax expression in the HTLV-1 infected cell line C81\textsuperscript{28,29}.
Depsipeptide inhibits both class I and class II HDAC including HDAC1\textsuperscript{30}; our data
suggested a similar action of depsipeptide in HTLV-1 infected cell lines.

**Definition of the maximum tolerated dose.** Different doses of depsipeptide (0.125, 0.25,
0.5, 1.0 and 2.0 mg/kg body weight 5 days/week for 2 weeks) were injected *i.p.* in the
NOD/SCID mice to determine the MTD. The mice injected with 2.0 mg/kg died (5 out of
5) during the injection course, and 80\% of the 1.0 mg/kg group mice died right after the
injection (day 14) indicating high toxicity at those doses. The groups of 0.5, 0.25 and
0.125 mg/kg survived for more than 6 months. Considering the tolerance of depsipeptide
in tumor bearing NOD/SCID mice might be lower than that in non-tumor bearing mice,
we choose to administer 0.5 mg/kg every other day for 2 weeks in the therapy study. It has
been reported in the literature that intraperitoneal administration of 0.5 mg/kg
depsipeptide three times a week is sufficient to inhibit histone deacetylase in mice\textsuperscript{31,32}.

**Effective Treatment of ATL with Depsipeptide alone and in combination with
daclizumab in the Met-I model.**

**The Small Tumor Burden Therapeutic Trial in Met-I model.**

In a therapeutic trial in the MET-1 model of human ATL, a two-week course of treatment
with depsipeptide (0.5 mg/kg every other day), a four-week course of treatment with
daclizumab (100ug/mouse, weekly) and the combination of depsipeptide with daclizumab
demonstrated therapeutic efficacy by both their effects on the serum levels of human
soluble Tac (sIL-2Rα) and soluble β2µ (Fig.3), and on the survival of leukemia-bearing mice (sIL-2Rα, 1,000 to 10,000 pg/ml) (Fig.4). When compared with the serum concentration of sIL-2Ra and β2µ in the PBS control group of mice at 8 weeks post therapy, there was a significant reduction of sIL-2Ra as well as β2µ levels in treated animals in depsipeptide group (p<0.0001), the daclizumab group (P<0.0001) and the combination of depsipeptide with daclizumab group (p<0.0001). Furthermore, there were significant reductions in human sIL-2Ra and reductions in β2µ levels that did not achieve significance in the combination therapy group of mice (depsipeptide plus daclizumab) when compared to that of the daclizumab alone group (sIL-2Ra, P<0.05; β2µ, P<0.1) or the depsipeptide alone group (sIL-2Ra, P<0.01; β2µ, P<0.08). The human sIL-2Rα and β2µ levels were undetectable in 9 out of 13 mice that received the combination of depsipeptide and daclizumab treatment when measured at 8 weeks post-therapy, suggesting those mice were virtually tumor-free. However, the leukemia progressed in those mice gradually as demonstrated by the increased human sIL-2Rα and β2µ levels when we terminated the experiment at day 200 post therapy (data not shown). The PBS control group mice died between day 48 and day 63. The control mice had extensive infiltrations of leukemic cells into a variety of organs including the lungs, liver and spleen. In contrast, the depsipeptide treatment alone (P<0.0001), daclizumab treatment alone (P<0.0001) and the combination of depsipeptide and daclizumab treatment (P<0.0001) had significantly prolonged survival of the leukemia bearing mice (Fig.4). Furthermore, the mice in the treatment groups had much less infiltration in the lungs, liver and spleen but had tumor outgrowth subcutaneously when examined at the endpoint. This may be due to incomplete saturation of the IL-2 receptor by daclizumab in the skin.
as compared to blood or lymph nodes where the receptor is easily saturated as observed with patients with ATL (Dr. Waldmann, unpublished data). All the mice in the PBS group died before day 63 post therapy. However, at 120 days post therapy, 2 out of 13 mice in the depsipeptide treatment alone and daclizumab treatment alone groups and 13 out of 13 mice in the combination therapy group were still alive. The combination of depsipeptide and daclizumab treatment significantly prolonged the survival of leukemia bearing mice when compared with depsipeptide treatment alone (P<0.001) or daclizumab treatment alone (P<0.0001). 7 out of 13 mice survived more than 200 days in the combination group (Fig. 4).

**The Larger Tumor Burden Treatment Trial in the Met-1 Model.**

The tumor burden was triple that of the small tumor burden group (sIL-2Ra, 10,000 to 25,000 pg/ml). The treatment of depsipeptide and daclizumab (same dose and dosing schedule as the small tumor burden trial) alone, and the combination of these two agents demonstrated therapeutic efficacy. The serum levels of tumor marker soluble IL-2Ra and β2μ in the depsipeptide group, daclizumab group and the combination group was significantly lower than that of the PBS group at 4 weeks post therapy (data not shown). All three treatments significantly prolonged the survival of tumor bearing mice (P<0.0001). Furthermore, the combination of depsipeptide with daclizumab significantly prolonged the survival of the tumor bearing mice when compared with depsipeptide alone (P<0.01) or daclizumab alone (P<0.05). Similar to the small tumor burden trial, the mice in the PBS group had extensive infiltrations of leukemic cells in the lungs, liver and spleen. While the mice in the treatment group had much less infiltration in the lungs, liver
and spleen but had tumor outgrowth subcutaneously. The mice in the PBS group died before day 45 post therapy. However, at day 100 post therapy, 1 out of 8 mice in the depsipeptide treatment alone group, 2 out of 8 mice in the daclizumab treatment alone group were still alive. Furthermore, 5 out of 8 mice in the combination therapy group survived more than 180 days (Fig. 5).

**Discussion**

The MET-1 ATL model presents many features that parallel those observed in patients with adult T-cell leukemia and thus represents a valuable model for the evaluation of the efficacy of therapeutic agents directed toward ATL. In earlier studies daclizumab showed efficacy both in the MET-1 ATL model and in human clinical trials. In the human study, therapy with the unmodified murine version of anti-Tac provided effective therapy for 6 of 19 patients with ATL studied. There are two modes of tumor killing manifested by the monoclonal antibody daclizumab. One is the blockade of the binding of IL-2 to its receptor (IL-2Rα), as shown in patients with smoldering ATL, therefore leading to cytokine-deprivation induced apoptosis. Another mode is antibody dependent cellular cytotoxicity (ADCC), as shown in murine Met-1 model, which requires the expression of FcRγIII on monocytes and granulocytes. Daclizumab provides no therapeutic efficacy in the murine ATL model when examined in FcRγ knockout (FcRγ-/-) mice that do not express FcRγIII. Recently, another mechanism of action has been reported. In particular, in humans the interaction of daclizumab with the IL-2 receptor alpha subunit was associated with a 4- to 20-fold increase in the number of circulating CD56bright, CD25 expressing, IL-10 secreting natural killer cells that mediate negative
immuno-regulatory actions. A number of approaches could be exploited to optimize the action of daclizumab in the therapy of leukemias and lymphomas. A paradigm is being established that monoclonal antibodies will not be used ultimately in monotherapy of human malignancy, but rather will be used in association with an array of agents including chemotherapeutic agents that manifest a different mode of action. In the MET-1 model, we have shown that the combination of daclizumab with the chemotherapeutic agent Velcade (bortezomib or PS-341, a proteasome inhibitor) or flavopiridol (a CDK inhibitor) had synergistic therapeutic effects in the treatment of ATL. To continue this theme, we are testing other chemotherapeutic agents such as HDAC inhibitors (i.e., depsipeptide) in combination with daclizumab in the Met-1 model.

The histone deacetylase inhibitors are a new class of antineoplastic agents currently being evaluated in clinical trials. Several families of HDAC inhibitors have been characterized. These include the short-chain fatty acids, such as sodium butyrate and valproic acid; the organic hydroxamic acids, such as trichostatin A (TSA) and suberanilohydroxamic acid (SAHA); the benzamides, such as CI-994 and MS-27-275; the cyclic tetrapeptides, such as trapoxin A; and the bicyclic depsipeptides, such as depsipeptide. Among them, Vorinostat (SAHA) has been approved by FDA as monotherapy for cutaneous T cell lymphoma. In addition, Depsipeptide has shown major responses in the treatment of leukemia and lymphomas. Similar to other HDAC inhibitors, depsipeptide has been shown to induce cell cycle arrest, cellular differentiation, and apoptosis. Although the precise mechanism of cell growth arrest, cellular differentiation and apoptosis is not clear; it is commonly accepted that depsipeptide exerts its anti-tumor effect via modulation of the expression and functions of cell cycle regulators and
apoptosis related molecules\textsuperscript{16}. The molecular changes induced by depsipeptde include increased expression of p21\textsuperscript{38}, altered expression of cyclins, hyperphosphorylation of Rb\textsuperscript{38}, and decreased expression of c-myc in fibroblasts and in a T-cell hybridoma\textsuperscript{39}. Consistent with this, we observed that depsipeptide induced increased expression of p21, decreased expression of cyclin A and decreased expression of anti-apoptotic proteins Bcl-2 and Bcl-X\textsubscript{L} in HTLV-I infected T-cell lines.

Besides its anti-tumor effects, depsipeptide has been shown to be able to induce the expression of IL-2R\textalpha, the target of daclizumab, both in cell lines\textsuperscript{21} and in the patients with T cell lymphoma\textsuperscript{15}. We reasoned that depsipeptide which was reported to be an Il-2R\textalpha inducing agent might augment the activity of daclizumab, the combination of depsipeptide with daclizumab may improve the efficacy of daclizumab in the treatment of ATL. Indeed, in our Met-1 model of ATL, we observed greater efficacy for the combination of depsipeptide with daclizumab. When administered \textit{i.p.} at 0.5mg/kg every other day for two weeks, the tumor growth was significantly inhibited and the survival of the mice was significantly prolonged in the combination group when compared with those in either the daclizumab or depsipeptide groups alone(Fig.4 &5). However, in our study depsipeptide treatment did not increase the expression of IL-2R\textalpha in HTLV-1 infected cell lines (data not shown). This suggested that the synergistic effect between depsipeptide and daclizumab was probably due to the combined effects of monoclonal antibody targeted therapy with a chemotherapeutic molecule, agents that act through different modes of tumor killing. As discussed above, the cytotoxicity of despeptide is induced by modulation of genes involved in cell cycle regulation and apoptosis\textsuperscript{40}. On the other hand, the monoclonal antibody daclizumab, does not act through blockade of the
IL-2/IL2Ra autocrine loop in the Met-1 ATL model since the leukemic cells do not produce IL-2, nor do they express IL-2 mRNA. In addition, they do not proliferate in response to murine IL-2. Depsipeptide treatment did not induce the expression of IL-2 either (data not shown). The immunoglobulin-Fc receptor does appear to be the major element involved in the action of daclizumab as daclizumab lost its therapeutic efficacy in the FcRγIII deficient mice. However, depsipeptide treatment had no effect on FcRγIII expression in NOD/SCID mice (data not shown). This suggests that the synergistic effect of depsipeptide with daclizumab was not due to increased FcRγIII expression.

Currently, depsipeptide is in the phase II trial with cutaneous and peripheral T cell lymphoma. Electrocardiogram abnormalities, thought to be a class effect associated with HADC inhibitors, were observed both in preclinical animal studies and in phase I testing of depsipeptide. However, extensive cardiac studies in the phase II trial of depsipetide in T cell lymphoma concluded that the administration of depsipeptide was not associated with myocardial damage or impaired cardiac function. The potential effect of heart rate-corrected QT interval prolongation remains under study. With significant clinical benefit in patients with cutaneous and peripheral T cell lymphoma, this safety data could further support the use of depsipeptide in patients with leukemias and lymphomas.

In conclusion, our data showed that depsipeptide had an anti-tumor effect both in vitro and in vivo. The combination therapy of depsipetide with daclizumab had significant synergistic therapeutic effect in the murine model of ATL. The results of our study
support a trial of depsipeptide in ATL patients, preferably, as an agent combined with daclizumab therapy.

**Acknowledgment**

This study was supported by the intramural research program of the National Cancer Institute, NIH. The authors declare no competing financial interests. All animal experiments were performed in accordance with National Institutes of Health Animal Care and Use Committee guidelines. J.C. designed and performed research; M.L.Z and W.J. helped with the animal study; T.A.W. designed the study and revised the paper.
Reference


Figure legends

**Fig.1** Depsipetide treatment inhibited the cell proliferation and induced apoptosis in **HTLV-I infected cell lines.** A) The cells were treated with various concentrations of depsipeptide (0, 0.12, 0.5, 2, 5ng/ml) and the cell proliferation was measured at 72hrs later by $^3$H thymidine incorporation. The data are shown as percentage of untreated control and represent mean ± SD of triplicates and are representative of three independent experiments. B) Induction of apoptosis by depsipeptide. The cells were treated with 2ng/ml depsipeptide for 24hrs or 48hrs and the apoptotic cells were measured by annexin V staining. Data represent the mean percentage of apoptotic cells from three independent experiments. C) Induction of caspase-3 and caspase-9 activities by depsipeptide. The data are representative of three independent experiments.

**Fig.2** Depsipeptide treatment induced accumulation of histone acetylation and altered the expression of cyclin A, p21, Bcl-2 and Bcl-XL in **HTLV-I infected cell lines.** A) Accumulation of histone H3 acetylation after depsipetide treatment. MT-2 and Hut102 cells were treated with 5ng/ml depsipeptide for 24hrs and 48hrs. Total cell lysate (50ug/lane) were separated on SDS-polyacrylamide gels and transferred to the membrane. Acetyl-histone H3 or histone H3 levels were detected by western blot with specific antibodies.  B) Effect of depsipetide on the expression of cyclin A, cyclin D, p21, Bcl-2, Bcl-XL and viral protein Tax. Cell lines were treated with 5ng/ml depsipeptide for 24hrs and 48hrs, and then total cellular protein was extracted and western blot analysis was performed.
The growth of MET-1 ATL cells in NOD/SCID mice bearing the MET-1 ATL leukemia was inhibited by daclizumab, depsipeptide and the combination of daclizumab with depsipeptide. MET-1 ATL cells were transferred into mice. The groups (13 mice/group) included those receiving PBS, 2-week every other day 0.5mg/kg/dose depsipeptide, 4 weekly doses of 100 µg daclizumab, the combination of 2-week every other day 0.5mg/kg/dose depsipeptide with 4 weekly doses of 100 µg daclizumab. A) the mean concentration of sIL-2Rα in picograms per milliliter. The animals treated in the 2-week depsipeptide, 4-week daclizumab, and the combination of 2-week depsipeptide with 4-week daclizumab groups had significantly decreased values of sIL-2Rα when compared with those of the PBS control group 8 weeks post therapy (P < 0.0001). Furthermore, the animals receiving the combination of depsipeptide with daclizumab had significantly decreased levels of sIL-2Rα when compared with those of the mice in the depsipetide alone group (P<0.01) and daclizumab alone group (P< 0.05). B) The mean concentration of β2µ in micrograms per milliliter 8 weeks post therapy. The serum levels of β2µ were significantly lower in the depsipetide treatment alone, daclizumab treatment alone, and combination group compared with PBS group (P<0.0001). The animals receiving the combination of depsipeptide with daclizumab had decreased levels of β2µ when compared with those of the mice in the depsipetide alone group (P<0.08) and daclizumab alone group (P< 0.1).

Figure 4. Depsipeptide and its combination with daclizumab prolonged the survival of Met-1 leukemia bearing SCID/NOD mice. At the time of the experiment, the mice
had sIL-2Rα levels of 1000 to 10,000 pg/ml. The groups (13 mice/group) included those receiving intravenous PBS, 0.5mg/kg/dose depsipeptide every other day for 2 weeks, 100 µg daclizumab weekly for 4 weeks, and a combination of 0.5mg/kg/dose depsipeptide every other day for 2 weeks with 100 µg daclizumab weekly for 4 weeks. Another group receiving no tumor and no therapy was set up as a life span control for NOD/SCID mice. Event-free survival was followed for 200 days. The animals treated in depsipeptide, daclizumab and the combination of daclizumab with depsipeptide groups had significantly prolonged survivals when compared with the PBS control group ($P < 0.0001$). The combination of depsipeptide with daclizumab significantly prolonged the survival of leukemia bearing mice when compared with depsipeptide alone ($P < 0.001$) or daclizumab alone ($P < 0.0001$).

**Figure 5. Depsipeptide inhibited the tumor growth and prolonged the survival of leukemia-bearing mice in the large tumor burden therapeutic study.** At the time of the initial therapy, the mice had sIL-2Ra levels of 10,000 to 25,000 pg/ml. Groups are the same as those described in Figure 4. The animals treated in depsipeptide, daclizumab and the combination of daclizumab with depsipeptide groups had significantly prolonged survivals when compared with the PBS control group ($P < 0.0001$). The combination of depsipeptide with daclizumab treatment significantly prolonged the survival of leukemia bearing mice when compared with depsipeptide alone treatment ($P < 0.01$) or daclizumab alone treatment ($P < 0.05$).
Fig. 1

A

Percentage of control proliferation

Hut102  CaGT  MT-2  MT-1  MJ

concentration

medium  0.12ng/ml  0.5ng/ml  2ng/ml  5ng/ml

Percentage of apoptotic cells

Hut102  CaGT  MT-2  MT-1  MJ

24hrs  48hrs

B

C

Fold induction

Hut102  CaGT  MT-2  MT-1  MJ

Caspase-3  Caspase-9

untreated  treated
Fig. 2

A

B

From www.bloodjournal.org by guest on October 24, 2017. For personal use only.
Fig. 3

A

Soluble Tac (pg/ml)

PBS        FK228     Daclizumab FK228 + Daclizumab

P<0.01

P<0.05

B

Serum β2µ level (µg/ml)

PBS        FK228     Daclizumab FK228 + Daclizumab

P<0.08

P<0.1
Fig. 4

- no tumor, no therapy
- PBS
- daclizumab
- FK228
- daclizumab + FK228

days after therapy

number of live mice
Fig. 5

The graph shows the number of live mice over days after therapy for different treatments:
- PBS
- Daclizumab
- FK228
- Daclizumab + FK228
Effective treatment of a murine model of adult T-cell leukemia using depsipeptide and its combination with unmodified daclizumab directed toward CD25

Jing Chen, Meili Zhang, Wei Ju and Thomas A Waldmann