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Safety, efficacy, and pharmacokinetics/pharmacodynamics of daclizumab (anti-CD25) in patients with adult T-cell leukemia/lymphoma

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Abstract

Interleukin-2 receptor α chain (CD25) is overexpressed in human T-cell leukemia virus 1 associated adult T-cell leukemia/lymphoma (ATL). Daclizumab a humanized monoclonal antibody blocks IL-2 binding by recognizing the interleukin-2 receptor α chain (CD25). We

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Authorship Contributions

JLB, TAW and JCM designed and performed research, performed patient care, analyzed and interpreted data and wrote the paper. JEJ, DMS, MT, GHW and NU performed patient care and collected patient samples. ESJ, MSS, TAF and CKG performed research. WDF, CJP, and JS performed data and statistical analyses. All authors had access to the primary clinical trial data.

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Disclosure of Potential Conflicts of Interest

None of the authors has any potential financial conflict of interest related to this manuscript.

conducted a phase I/II trial of daclizumab in 34 patients with ATL. Saturation of surface CD25 on circulating ATL cells was achieved at all doses; however saturation on ATL cells in lymph nodes required 8 mg/kg. Up to 8 mg/kg of daclizumab administered every 3 weeks was well tolerated. No responses were observed in 18 patients with acute or lymphoma ATL; however, 6 partial responses were observed in 16 chronic and smoldering ATL patients. The pharmacokinetics / pharmacodynamics of daclizumab suggest that high-dose daclizumab would be more effective than low-dose daclizumab in treatment of lymphoid malignancies and autoimmune diseases (e.g., multiple sclerosis) since high-dose daclizumab is required to saturate IL-2R alpha in extravascular sites.

Keywords

Adult T-cell leukemia/lymphoma; daclizumab, human T- cell leukemia virus 1 (HTLV-1) associated ATL; interleukin-2 receptor-alpha; monoclonal antibody

[1] INTRODUCTION

Adult T-cell leukemia/lymphoma (ATL) is an uncommon and aggressive lymphoproliferative disorder resulting from infection with the retrovirus, human T-cell lymphotropic virus type 1 (HTLV-1) [1-3]. The disease clusters within certain geographical regions that include southwestern Japan, the Caribbean Basin, Northeastern South America, Sub-Saharan Africa, as well as pockets in the African-American population in the Southeastern United States [4]. In general, ATL is associated with a poor prognosis and effective therapy is limited (for review, please see Tsukasaki, et al) [5]. ATL is usually classified into 4 clinical subtypes: acute, lymphomatous, chronic and smoldering [6]. The acute and lymphomatous subtypes exhibit a clinically aggressive course with median survivals of 4 to 10 months, while chronic and smoldering subtypes are more indolent. A study in a predominantly Caribbean population reported median survivals of 17 and 34 months, respectively in these two subtypes [7].

ATL is characterized by the presence of increased numbers of malignant CD4+ T-cells in the peripheral blood, lymph nodes and infiltration of other organs. ATL cells overexpress CD25, the 55-kDa alpha chain of the interleukin-2 receptor (IL-2R α , TAC). In contrast, with the exception of Tregs, CD25 is typically only expressed at low levels on normal circulating T-cells [8], making it a potential target for therapy. In the early phases of ATL, expression of the viral *Tax* gene induces T-cell proliferation through the induction of a number of cytokine-cytokine receptor autocrine growth stimulation loops including IL-2/IL-2R α -induced proliferation [9]. Anti-Tac, a murine monoclonal antibody that binds human CD25 and blocks IL-2 binding, not only inhibits tumor cell proliferation, but is also postulated to produce cytokine deprivation and antibody-dependent cellular cytotoxicity (ADCC) mediated apoptotic cell death. Studies in the MET-1 xenograft mouse model of human ATL showed daclizumab, a humanized version of anti-Tac, inhibited tumor growth and improved survival [10, 11].

Waldmann and colleagues demonstrated the clinical antitumor activity of murine anti-Tac in patients with refractory ATL with six of nineteen patients achieving a response [10]. Murine

anti-Tac use was limited however because it is a non-human antibody. Murine anti-Tac is immunogenic with three of the responders developing human anti-mouse antibodies (HAMA) preventing further treatment. In addition, the serum half-life of the murine antibody was short at about 40 hours, limiting its ability to achieve long-term saturation of CD25 and blockade of IL-2 binding to ATL cells. In 1998, the anti-CD25 antibody, daclizumab (Zenapax®, Roche, Nutley, NJ) became available [12-14]. Daclizumab is a humanized monoclonal antibody generated using recombinant DNA technology that was approved for the prophylaxis of acute allograft rejection in patients receiving organ transplants [14-15].

We conducted a phase I/II study in which up to 8 mg/kg of daclizumab was administered on a 3-week schedule to patients with ATL. The scientific hypothesis forming the basis for this study was that the survival of ATL cells is dependent on an IL-2/IL-2R alpha autocrine growth loop and that daclizumab could block this loop. The objectives of the study were to determine the daclizumab dose required to achieve 95% saturation of CD25 targets on the surface of ATL cells in the peripheral blood and lymph nodes, and to maintain this level of saturation between treatment cycles. Additional endpoints included the assessment of adverse events associated with high-dose daclizumab treatment and the determination of the antitumor activity of this treatment in the different subtypes of ATL.

[2] METHODS

[2.1] Study design and objectives

This was an NCI-IRB approved, single institution, open-label phase I/II study (clinicaltrials.gov: NCT00020020) performed at the Clinical Center of the National Cancer Institute in Bethesda, MD. All studies were approved by the IRB of the NCI, NIH and the NCI Ethics Committee and were performed in accordance with the 1964 Declaration of Helsinki and its later amendments. All persons gave their written informed consent prior to their inclusion in the study. In phase I, daclizumab (Zenapax®, Roche, Nutley, NJ) was administered to groups of ATL patients in a 3 + 3 dose-escalation design. Phase I endpoints included: (1) the determination of serious adverse events associated with saturating doses of daclizumab, the definition of the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD) of daclizumab in ATL, (2) determination of the dose of daclizumab required to achieve 95% saturation of surface CD25 on ATL cells in the peripheral blood and lymph nodes, and to maintain this saturation between treatment cycles, and (3) determination of the pharmacokinetics of high-dose daclizumab.

[2.2] Patient eligibility

Patients were required to have a pathologically confirmed diagnosis of ATL and have serum antibodies to HTLV-1. At least 5% of their ATL cells must react with anti-CD25 by immunofluorescent staining on a paraffin section, or on flow cytometry of peripheral blood, lymph node or bone marrow aspirate, or their serum soluble IL-2 alpha receptor (sCD25) level must be 1,000 units/mL. All ATL subtypes were eligible, and patients were required to have measurable disease. Patients were required to be 10 years of age, have a life expectancy >2 months, a Karnofsky performance score >60%, a granulocyte count of

500/mm³ and a platelet count of 25,000/mm³. Patients were also required to have a serum creatinine of 2.9 mg/dL, a bilirubin <2.9 mg/dL, SGOT and SGPT 5.0 × the upper limit of normal, and able to provide informed consent. Exclusion criteria included cytotoxic chemotherapy within 3 weeks of study entry, the presence of human antihuman antibodies (HAHA) [16] to daclizumab, symptomatic involvement of the central nervous system, pregnancy, or nursing, and HIV-positive patients.

[2.3] Patient evaluation

Patients were assessed by history, physical exam, laboratory studies and flow cytometry at baseline and prior to initiation of each treatment cycle. ATL cells in the peripheral blood or bone marrow were detected and counted as an aberrant population of CD3⁺/dim/CD4⁺/CD25⁺ T-cells using flow cytometry. Patients were also assessed using computerized tomography at baseline and prior to treatment on days 36 and 99. Skin involvement was scored using the Severity- Weighted Assessment Tool (SWAT) [17].

Previously we published [18, 19] analyses of the impact of soluble TAC (IL-2R alpha) on the bindability of administered anti-IL-2R alpha and on the impact of tumor burden on antibody dosing. There we indicated that in situations such as with high specific activity radiolabeled antibody one must use the soluble circulating IL-2R alpha levels to provide the optimized dose of unlabeled daclizumab. However as is true in the present case for routine clinical use of unlabeled daclizumab, because there is no dose-related toxicity of the antibody, our alternative strategy in the present manuscript is to bypass complex types of analyses altogether and to administer very large doses (e.g. 8 mg/kg) that overcome variations in sIL-2R alpha concentrations and in tumor CD25 burden.

[2.4] Treatment

Patients treated at the first dose level (cohort 1) received intravenous daclizumab 2 mg/kg over 60 minutes on days one and two. Subsequent cohorts (2, 3 and 4) received daclizumab 4, 6 or 8 mg/kg, respectively administered over 90 minutes as a single dose on day one. In the absence of DLT or disease progression, patients received five additional courses of daclizumab at the same dose on days 15, 36, 57, 78 and 99. Patients on phase II were treated at the saturating dose determined in phase I. If saturating doses were not achieved by the highest planned phase I dose, then the highest daclizumab dose (8 mg/kg) was studied in phase II. Patients on both phases that achieved partial responses after 6 doses in the absence of serious toxicity were allowed to continue on daclizumab treatment at the same dose every 3 weeks until progression.

[2.5] Adverse events and responses

Adverse events were categorized and graded using the NCI Common Toxicity Criteria v3.0, the version then active at study initiation. DLT was defined as any of the following attributable to daclizumab treatment: (1) any grade 3 or greater non-hematological toxicity, except an allergic reaction or alopecia, (2) grade 2 or greater allergic toxicity, ataxia or seizures, or (3) grade 4 hematological toxicity. The MTD was defined as the highest dose of daclizumab at which less than two patients experienced a DLT.

Responses were determined using modified WHO response criteria [20]. A complete response (CR) was defined as disappearance of all measurable and non-measurable disease lasting more than one month. A partial response (PR) was defined as a reduction of 50% in the circulating leukemia cell count, or 50% reduction in the sum of measurable lesions and no increase in size of any measurable or non-measurable lesion, or the appearance of a new lesion for at least one month. Progressive disease (PD) was defined by 25% increase in leukemic cell count, the appearance of any new lesion, or an increase of 25% in any measurable disease. Patients who did not meet the criteria were classified as stable disease (SD).

[2.6] Correlative laboratory studies

Daclizumab pharmacokinetic (PK) studies were performed on patients treated in phase I. Serum samples were collected from patients relative to the day 1 infusion at: pre-dose 10 min, 2, 16, 24, 48, 96 hr, and 336 hr (day 14).

Non-compartmental analysis, using the linear-log linear trapezoidal rule to integrate the area under the concentration-time curves and weight-normalized doses (mg/kg), was initially performed on patients (n = 14) given a single dose of daclizumab at four dose levels (2, 4, 6 and 8 mg/kg). Pharmacokinetic analysis was limited to 14 days after the first dose, just prior to the administration of the second dose. Serum concentrations of daclizumab were assessed by ELISA, with a lower limit of quantification of 300 ng/mL, as previously described [21]. All patients on the 2 mg/kg dose level (n = 3) were administered an additional infusion 24 hours into the first 14-day interval.

A three compartment pharmacokinetic structural model, based on a previous structural model [19], was applied to this study that factored in between- and within-subject variability and available covariate (soluble TAC, body weight and WBC). The disposition of daclizumab is represented by the following equation: $C_p = A e^{-at} * B e^{-bt} * C e^{-ct}$, where A, B, and C are related to the size of the plasma, tissue, and target (i.e. CD25) tumor compartments. Patients with a small tumor burden, as measured by sTAC, have a small, almost negligible $C e^{-ct}$ parameter, thus disposition is described with essentially two-compartment. Patients with large tumor burdens have a $C e^{-ct}$ parameter that produced a three-compartment disposition. Patients with extremely large tumor burden have a $C e^{-ct}$ parameter that is much greater than the $A e^{-at}$ or $B e^{-bt}$ parameters that their disposition will essentially follow a pseudo-one compartment disposition and the $T_{1/2}$ will be short.

The saturating dose of daclizumab was defined using flow cytometry as the dose at which 6 of 6 patients demonstrated 95% saturation of surface CD25 on ATL cells in the peripheral blood and lymph nodes 6 to 72 hours after the initial daclizumab dose, and that this saturation was maintained at days 15 and 36 prior to the second and third doses respectively. Phase II objectives were to estimate the response rate to saturating doses of daclizumab in ATL as well as the duration of response and overall survival. Saturation analysis of CD25 was performed on ATL cells in the peripheral blood and on tumor cells obtained by fine-needle aspiration of palpable lymph nodes or masses using flow cytometry. Analyses were performed prior to the first daclizumab infusion, again at 6 to 72 hours after the first infusion, and prior to the daclizumab infusions on days 14 and 36 (cycles 2 and 3). Indirect

immunofluorescent staining was performed using fluorescein isothiocyanate (FITC)-labeled antihuman CD3 (BD Biosciences, Franklin Lakes (NJ)), peridinin chlorophyll proteins (PerCP) labeled antihuman CD4 (BD Biosciences). Direct immunofluorescent staining to define saturation was performed with phycoerythrin PE-labeled antihuman CD25 (BD Biosciences, daclizumab), and PE-labeled 7G7/B6 monoclonal antibody (Ancell, Bayport, MN), that recognizes a non-competing epitope on CD25 [22]. Leukemic cells were deemed to be saturated when the reaction of the cells with fluorochrome-labeled daclizumab in direct immunofluorescence analysis was reduced by 95% when compared to pretherapy reactivity or when compared to the reactivity with the PE-labeled 7G7/B6 monoclonal antibody.

[2.7] Statistical analysis

The phase II part of the study was designed using a Simon two-stage design with a goal response rate of 30% [23]. Kaplan-Meier curves for overall survival and progression-free survival were estimated using R Project for Statistical Computing [24]. Statistical significance for subgroup comparison was calculated using the Fisher's exact test. A p-value of <0.05 was considered significant.

[3.0] RESULTS

[3.1] Patients

Thirty-four patients were registered and treated on the study between December 1999 and December 2009. All patients received at least one treatment. A total of 250 treatment cycles were administered with a median of 4.5 cycles administered per patient (range, 1-75 cycles). Fourteen patients were included in phase I, and 20 were treated in phase II. The phase II patient characteristics are shown in Table 1. Most were African-Caribbean, had received prior therapy for their ATL, and had a Karnofsky performance status 70%. Phase I patients received a greater number of prior treatments (median two vs. one prior therapies); a higher proportion of phase I patients had received prior chemotherapy (64% vs. 35%), and phase I had a greater number of patients with the acute or lymphoma subtypes (72% vs. 40%). This latter difference is due to an amendment to the phase II protocol to include only patients with the chronic and smoldering subtypes after the first 9 patients were treated.

[3.2] Pharmacokinetics

Pharmacokinetic studies were performed in the phase I portion of the study (Table 2, Figure 1) using dose levels 4, 6, and 8 mg/kg only. The 2 mg/kg dose level (n=3) was excluded from analyses due to the additional doses given one day after the first dose at this dose level, making this dose level incomparable to the others (n=11). The half-life of daclizumab was estimated to be between 3.0 to 7.8 days. There was a more than dose-proportional increase, albeit non-significant ($P=0.267$; Kruskal-Wallis test), in dose-normalized AUC, suggesting nonlinear pharmacokinetics. Daclizumab volume (L) and clearance (mL/hr) were not statistically different between dose levels ($p=0.3338$ and $p=0.2667$, respectively, Kruskal-Wallis). Mean volume $\pm$ 1.68L, clearance (43.65 ± 30.82 mL/hr), and half-life (4.23 ± 2.94 days) values, regardless of dose, were consistent with literature values of 7.3 L, 57 mL/hr, and 4.12 days.

The three-compartment pharmacokinetic linear model was tested with soluble TAC (sTAC), body weight, and WBC as potential covariates, and only sTAC as a covariate on volume of distribution significantly improved the model (Figure 2). This was further supported by the observed trend ($r^2=0.1828$), albeit nonsignificant ($p=0.1451$), between daclizumab V_{ss} (from noncompartmental analysis) and sTAC levels (Figure 2c) that dose-normalized AUC, suggesting nonlinear pharmacokinetics. Conversely, daclizumab clearance (CL) was slightly, but non-significantly decreased at higher doses from 0.67 mL/hr/kg at 4 mg/kg to 0.40 mL/hr/kg at 8 mg/kg. The mean daclizumab concentrations at day 14 increased between the 4 mg/kg to 6 mg/kg treatment groups, and again at the 8 mg/kg dose.

[3.3] Daclizumab pharmacodynamics, CD25 saturation

Saturation of surface CD25 on ATL cells by daclizumab was measured in the peripheral blood of all 34 patients and in the lymph nodes of 6 lymphomatous ATL subtype patients. Flow cytometry demonstrated saturation of CD25 by daclizumab on circulating ATL cells (Figure 3). In this patient, shown in Figure 3, 95% saturation was achieved 24 hours after the initial dose of daclizumab that was maintained for 2 weeks after the first dose, and for 3 weeks after the second daclizumab dose. Thirty-three patients (97.1%) had their circulating ATL cells saturated 6-72 hours after the initial dose of daclizumab, and all 20 patients receiving 4 to 8 mg/kg achieved 95% saturation within the first two treatment cycles. In 6 patients who underwent fine-needle aspiration of their lymph nodes, the percentage that demonstrated and maintained saturation was considerably less. Only 3 of 6 patients manifested saturation of the CD25 on their ATL cells in lymph nodes. At the initial dose of 2 mg/kg given on days one and two 1 of 2 patients' lymph nodes demonstrated saturation. In the other patient 55% of these cells were saturated. In the 6 mg/kg cohort the saturation in one patient's lymph nodes was not maintained. Saturation of the lymph nodes was observed in the 8 mg/kg cohort. No DLT was observed, therefore, 8mg/kg was the dose selected for study in phase II.

[3.4] Adverse events

Overall, daclizumab was well tolerated with the majority of adverse events reported being grades 1 or 2. No dose-limiting toxicity was attributable to therapy. In phase I eight grade 3 or 4 non-hematological adverse events occurred, all of which were unlikely related, or unrelated to therapy. Grade 3 toxicities included one soft-tissue infection, one episode of psoriasis, one episode each of hyponatremia, hypercalcemia or hypokalemia, as well as two occurrences of hypophosphatemia. The only grade 4 toxicities were one episode each of hypercalcemia and congestive heart failure. The patient who developed heart failure had previously received doxorubicin-based therapy. She was found to have dilated cardiomyopathy after 11 cycles of daclizumab and died 21 months later of congestive heart failure (CHF) with active ATL.

Twelve grade 3 or 4 non-hematological adverse events were reported in phase II (Figure 4). The only grade 4 toxicity was hyperglycemia, which was not attributed to daclizumab. Most of the grade 3 toxicities were scored as unlikely related or unrelated to therapy. These included one episode each of dyspnea, wheezing, bronchial obstruction, hyperglycemia, hypercalcemia, hyponatremia or elevated prothrombin time. The remaining four adverse

events of hyperbilirubinemia, hyperamylasemia, congestive heart failure and the development of acute psoriasis were scored as possibly attributable to daclizumab. The patient with heart failure was 80-years old and had received 8 cycles of daclizumab. She had prior diagnoses of hypertension and atrial fibrillation, and had an aortic-valve replacement. She died 4 months later of CHF. The patient that developed acute generalized psoriasis did so after 26 cycles of daclizumab. It resolved with a course of corticosteroid and acitretin (Soriatane) treatment. He is currently alive and well, and in complete remission more than 3 years after his last dose of daclizumab.

[3.5] Response and survival

Patient responses were evaluated using physical examination, standard laboratory studies, computerized tomography, semi-quantitative assessment of skin disease and measurement of circulating leukemic cells by flow cytometry. Nearly all patients had circulating ATL cells as measured by the number of CD4+/CD25+ cells as measured by the monoclonal antibody 7G7/B6 in the peripheral blood. In phase I, eight patients (57%) demonstrated a greater than 50% decrease in their circulating ATL cells. In phase II, 12 patients (66%) showed a greater than 50% decrease in their circulating ATL cells with treatment. The best response in circulating CD4+/CD25+ leukemic cells of each patient in phase II is shown in a waterfall plot (Figure 5). Despite these decreases in the number of circulating leukemic cells, most patients did not meet response criteria because either the decrease was not sustained or they showed disease progression at other sites.

Two phase I patients with the smoldering/chronic subtypes of ATL had partial responses. In phase II among the first 9 patients, of which only one had indolent ATL, there were no responses. Since 2 of 4 chronic/smoldering ATL patients responded to daclizumab in phase I and none of the 18 aggressive ATL patients from both phases I and II responded, the study was amended to include only patients with smoldering or chronic ATL (Figure 6). Of the 11 additional patients with smoldering or chronic ATL accrued to the study, there were 4 partial responses. The median duration of response in these 4 patients was 114 weeks (range, 21-220+ weeks) with 2 of the responses still ongoing for greater than 4 years at the time of this analysis. One patient that received daclizumab for 75 cycles was taken off of therapy due to unavailability of the drug. Within 3 months of discontinuation his disease progressed in the blood and skin. For phase II, the overall response rate was 20%. When we compared the response rates between aggressive (acute and lymphomatous subtypes) and indolent (chronic/smoldering subtypes) ATL patients treated at 8 mg/kg (20 phase II patients + 3 phase I patients), 4 out of 12 patients with indolent ATL had a response to daclizumab compared to none of the 11 patients with the aggressive subtypes of ATL ($p=0.093$). Since the ability to achieve the saturation of CD25 on leukemic cells in the peripheral blood was a primary objective of the study, and circulating ATL cells were saturated at all doses studied, and all responses were in circulating cells, we also compared the response rates between the two subgroups using the combined cohorts. For all 34 patients, the overall response rate was 18%. However, 6 of 16 patients with indolent ATL had a response to daclizumab compared to none of the 18 patients with the aggressive subtypes of ATL ($p = 0.006$).

All patients on the study were included in the analysis for overall survival (OS). One patient in phase II was excluded in the analysis of progression-free survival (PFS) due to the development of antibodies (HAHA) against daclizumab after a single treatment. Kaplan-Meier curves for PFS and OS for each phase are shown in Figure 5. For the 14 phase I patients, the median PFS and OS were 5.4 weeks (95% CI, 3.1–41.4) and 51.9 weeks (95% CI, 21.9–127.9), respectively. In phase II the median PFS and OS were 12 weeks (95% CI, 5.4–25.0) and 132.6 (95% CI, 53.9—not reached) weeks, respectively.

In light of the different outcomes of aggressive (acute and lymphomatous subtypes) and indolent (chronic/smoldering subtypes) ATL, we performed subgroup analyses of all patients treated at 8 mg/kg (Figure 5). For all 23 patients the median PFS and OS were 12 weeks (95% CI, 5.4–14.9 weeks) and 75 weeks (95% CI, 47.3–150.4 weeks) respectively. By subtype, the median PFS for aggressive ATL was 7.2 weeks (95% CI, 5.3–14.7 weeks) and the median OS in aggressive ATL was 53.9 weeks (95% CI, 26.6–142.1 weeks). The median PFS for indolent ATL was 14.0 weeks (95% CI, 6.6–14.7 weeks) and the median OS was not reached.

[4.0] DISCUSSION

Adult T-cell leukemia/lymphoma is a lethal lymphoproliferative disorder that results from chronic infection with the delta retrovirus, HTLV-1. Current therapy of ATL has demonstrated limited benefit. A randomized trial reported by the Japan Clinical Oncology Group showed an improved complete response rate with an intensive multidrug regimen, VCAP-AMP-VECP, compared to biweekly CHOP chemotherapy; however, overall survival was not significantly improved by the more intensive chemotherapy [25]. A recent meta-analysis suggested the combination of interferon-alpha and the antiretroviral agent zidovudine provided benefit in the leukemic subtype of ATL [26]. For 10–15% of patients diagnosed with the chronic/smoldering types of ATL therapeutic options are less clear. Few studies have focused on these subtypes of patients due to their relative infrequency and the general sense by many oncologists that the toxic effects of chemotherapy outweigh the benefit in these patients due to their more limited symptoms and longer survivals.

In the current study, we examined the pharmacokinetics, pharmacodynamics, safety and efficacy of an IL-2 receptor targeted therapy using high doses of the unmodified humanized anti-CD25 monoclonal antibody, daclizumab, in all Shimoyama subtypes of ATL. These pharmacokinetic studies demonstrated nonlinear (more than dose-proportional) pharmacokinetics, which are typical of monoclonal antibodies. The decreased clearance with dose, associated with an increased dose-normalized exposure (although neither statistically significant) suggested saturation of receptor binding. The daclizumab half-time estimated in the current study, 3.0 to 7.8 days, was shorter than the previously reported values of 11 to 20 days in which a dose of 1 mg/kg was administered [14].

The shortened half-time observed in the present study compared to those observed in patient populations with a low expression of tissue CD25 was highly dependent on the antigen expression per malignant cell and the total number of CD25+ cells reflecting the features of an “antigen sink” that absorbs out large quantities of the administered daclizumab. Given the

short half-life of survival it would appear to be more appropriate to have a shorter dose interval of 2 weeks rather than 3 weeks as was true in the present study. In accord with the recommendation of Ekberg H and coworkers [27] we suggest that a nadir daclizumab concentration of 3-7 ng/mL be maintained to provide the continuous blockade of CD25 (IL-2R alpha) required for daclizumab action.

The effective action of daclizumab depends on its saturation of CD25 thereby preventing the interaction of IL-2 with the high-affinity receptor and decreasing IL-2-mediated maintenance of the cytokine-dependent target cells. Saturation of CD25 on ATL cells was measured in the peripheral blood of all the 34 patients and in lymph nodes of 6 patients. Thirty-three of the 34 patients had their circulating ATL cells saturated 6-72 hours after the initial dose of daclizumab. In the 6 patients who underwent fine-needle aspiration of their lymph nodes the percentage that demonstrated and maintained saturation was considerably less. At 2 mg/kg only one of two patients demonstrated saturation. Lymph node ATL saturation was most consistently maintained at an 8 mg/kg cohort, therefore this dose was selected for study in phase II.

Daclizumab was well tolerated and demonstrated activity primarily in the chronic/smoldering subtypes of ATL. All adverse events occurred at daclizumab doses >2 mg/kg and all except one of these patients received extended dosing, suggesting that either one or a combination of these factors may explain their previously unreported toxicities. Since both ATL cells and Tregs express CD4 and CD25, it was not practical to define if high-dose daclizumab administration was associated with a reduction in normal Tregs. All the patients that developed autoimmune phenomenon also achieved prolonged disease free intervals after daclizumab treatment that could lead to the hypothesis that these patients also developed an immune response against their ATL as well.

Two patients experienced congestive heart failure while on study. This included Patient 07 in phase I and Patient 12 in phase II. Both had significant pre-existing cardiac risk factors. The first patient's episode of congestive heart failure was not initially attributed to daclizumab treatment; however a recent study has suggested an association between dysregulation of CD25+ T cells and congestive heart failure suggesting a potential mechanism that daclizumab might induce cardiac toxicity [28]. Another grade 3 toxicity, the acute onset of generalized psoriasis occurred in Patient 13 that was the only autoimmune event seen in this study. Interestingly, a twin of this patient under a "study exemption" who was treated off study with daclizumab on the study developed the acute onset of disseminated sarcoidosis requiring immunosuppressive therapy.

Cardiac toxicity and autoimmune events have not been reported in patients receiving prophylactic daclizumab during renal autograft transplant [27, 29]. In the organ transplant setting daclizumab is typically administered at 1 mg/kg for 5 doses in association with other immunosuppressive agents. Studies using extended daclizumab therapy have also not demonstrated cardiac toxicity or autoimmune events [30-33].

The antitumor activity of daclizumab appears to be restricted to patients with the chronic/smoldering subtypes of ATL where 37% of the patients responded. This observation is in

accord of studies of 6-day cultures of *ex vivo* cells from patients with various subtypes of ATL [9, 34-37]. The *ex vivo* PBMCs from patients with chronic and smoldering subtypes of ATL manifested spontaneous proliferation that could be inhibited by the addition of daclizumab. In contrast in virtually all the PBMCs of patients with acute ATL did not proliferate *ex vivo* and any proliferation observed was not inhibited by the addition of daclizumab suggesting that in only smoldering and chronic subtypes of ATL is there a persistent critical autocrine IL-2/IL-2 receptor system functioning [9, 34-37]. In patients with these indolent forms, median survival was not reached by >2 years of follow-up. Two patients with smoldering ATL treated on phase II achieved responses that lasted >4 years. Two other patients with chronic ATL in phase I also survived >5 years. Patient 03 with smoldering ATL experienced a 73% decrease in his circulating ATL cells after 3 cycles of daclizumab. However, likely as a result of a delay of cycle 4 this patient's ATL count increased in the interim and his response was unconfirmed. Patient 09 on phase I who had chronic ATL also achieved stable disease after 6 cycles of daclizumab with a 65% decrease in his circulating ATL cells. He received one additional dose of daclizumab after which he declined further treatment. At the time of analysis he was still alive >9 years after treatment.

In patients with the acute or lymphomatous subtypes of ATL there were no responses and the median overall survival of these patients treated at 8 mg/kg was 53.9 weeks; while this is greater than the 4 to 10 months reported in the literature [7] the confidence intervals were as low as 6 months. This lack of efficacy in these groups may be multifactorial, including the inability of daclizumab to consistently achieve or maintain saturation of CD25 on ATL cells in lymph nodes. This may be one explanation as to why no patients with lymphomatous ATL responded. Another factor may be that in these aggressive subtypes of ATL there is significant IL-2 independent growth of tumor cells [9, 34-37]. We have reported studies of the 6-day *ex vivo* culture of PBMCs from patients with chronic/smoldering and acute ATL [9]. In general, only two of nine PBMC samples from patients with acute ATL proliferated spontaneously and in these cases the *ex vivo* proliferation could not be inhibited by the addition of daclizumab. The PBMCs of 11 patients with chronic/smoldering ATL proliferated *ex vivo* and were associated with an IL-2/IL-2R alpha autocrine loop. However, they also manifested an additional autocrine IL-15/IL-15R alpha and a paracrine IL-9 stimulation pathway [34-37]. Although these latter two cytokines signal through the common gamma chain, JAK1, JAK3 and STAT5, their action is not affected by the addition of daclizumab that specifically targets IL-2R alpha. The IL-2 independence of acute ATL may involve c-Met, a receptor tyrosine kinase for hepatocyte growth factor, that has been found to be overexpressed in acute, but not chronic or smoldering ATL and IL-2 dependent ATL cell lines [38].

We have demonstrated that daclizumab is effective in the treatment of multiple sclerosis with an over 70% reduction in new or expanding MRI lesions in patients receiving this monoclonal antibody [39-42]. This efficacy was paralleled by a significant inhibition of the annualized relapse rate by over 50%. Mechanistic studies in multiple sclerosis demonstrated that a mode of action of daclizumab was the dramatic 6 to 8-fold expansion of immunoregulatory CD56^{bright} NK cells which gain access to the intrathecal compartment in MS and kill autologous activated T cells [41, 42]. Furthermore, daclizumab blocks trans-

presentation of IL-2 by mature dendritic cells to prime T cells resulting in inhibition of generation of antigen specific T cells [43]. In an industry sponsored placebo-controlled trial (CHOICE) study involving 230 patients with MS subcutaneous administration at high dose decreased new lesions by 72% but only by 25% in patients receiving low-dose daclizumab [39]. Both low-dose (1 mcg/kg every 4 weeks) and high-dose (2 mcg/kg every 2 weeks) daclizumab were sufficient to saturate the IL-2 receptor alpha on circulating cells for 4 weeks. However as shown by the present study, high-dose daclizumab is more effective at saturating and maintaining saturation of IL-2R alpha (CD25) in extravascular sites such as lymphoid organs in which antigen presentation occurs. The pharmacodynamic observations in the present study also explain the apparent paradoxical observation that efficacy of daclizumab in MS decreases 4 weeks after the intravenous dose despite the fact that the CD25 epitopes remain almost completely saturated (95%) in the blood [39].

We have shown that daclizumab, which targets CD25, has antitumor activity with the potential for achieving long-term responses in patients with the indolent forms of ATL. Targeted therapy using monoclonal antibodies armed with radionuclides or toxins directed at cell-surface molecules overexpressed in ATL cells offers another therapeutic option. Waldmann and colleagues reported a 57% response rate in 16 ATL patients treated with systemic radioimmunotherapy using yttrium-90 (⁹⁰Y)-labeled murine antihuman CD25 (TAC) [44]. Furthermore, having demonstrated that CD25 is a valid target in ATL, future directions might include the use of other therapies targeting the IL-2 receptor and its signaling pathway such as denileukin diftitox or LMB-2, a ScFv-Pseudomonas exotoxin recombinant protein. Responses of ATL to LMB-2 have been reported [45]. It is critical to recognize, as stated above, that in addition to the IL-2/IL-2 receptor activation by HTLV-1 Tax in patients with chronic/smoldering ATL, there is one additional autocrine IL-15/IL-15R and a paracrine IL-9 pathway [34-37]. Therefore, daclizumab that only blocks the IL-2/IL-2R pathway may not be sufficient. However, IL-2, IL-9 and IL-15 share the γ c receptor, JAK1, JAK3 signaling pathway. Therefore, the use of a JAK1 or JAK3 inhibitor could block this shared pathway used by γ c cytokines and thereby might provide effective therapy. In that regard, we have demonstrated the efficacy of the pan JAK inhibitor tofacitinib (CP-690, 550) and of the JAK1/JAK2 inhibitor ruxolitinib in inhibiting the *ex vivo* proliferation of smoldering/chronic ATL cells [37]. To translate these observations we have initiated a phase II clinical trial of ruxolitinib in patients with smoldering/chronic ATL.

[5.0] Conclusions

The interleukin-2 (CD25) receptor is overexpressed on ATL leukemia/lymphoma cells. Daclizumab that blocks IL-2 binding to CD25 on ATL cells has been associated with effective clinical responses in patients with indolent disease. Furthermore, it may also function to prevent indolent disease from transforming into aggressive ATL. Eight mg/kg of daclizumab were required to achieve 95% saturation of CD25 on ATL cells in lymph nodes. The pharmacodynamics of daclizumab provide a rationale for high-dose treatment in autoimmune diseases such as multiple sclerosis where it has been shown to provide effective therapy.

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Abbreviations

ADCC	antibody-dependent cellular cytotoxicity
ATL	adult T-cell leukemia/lymphoma
DLT	dose-limiting toxicity
FITC	fluorescein isothiocyanate
HAHA	human anti-human antibodies
HAMA	human anti-mouse antibodies
HTLV-1	human T-cell lymphotropic virus type 1
IL-2	interleukin-2
IL-2Rα	interleukin-2 receptor alpha
MTD	maximum tolerated dose
PE	phycoerythrin
PerCP	perdinin-chlorophyll proteins
PK	pharmacokinetics
SWAT	Severity-Weighted Assessment Tool

References

1. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood*. 1977; 50:481–92. [PubMed: 301762]
2. Gallo RC, Mann D, Broder S, et al. Human T-cell leukemia-lymphoma virus (HTLV) is in T but not B lymphocytes from a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci USA*. 1982; 79:5680–3. [PubMed: 6982476]
3. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci USA*. 1982; 79:2031–5. [PubMed: 6979048]
4. Broder S, Bunn PA, Jaffe ES, et al. T-Cell Lymphoproliferative Syndrome Associated with Human T-Cell Leukemia/Lymphoma Virus. *Annals of Internal Medicine*. 1984; 100:543–57. [PubMed: 6322632]
5. Tsukasaki K, Hermine O, Bazarbachi A, et al. Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: a proposal from an international consensus meeting. *J Clin Oncol*. 2009; 27:453–9. [PubMed: 19064971]
6. Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma. A report from the Lymphoma Study Group (1984-87). *Br J Haematol*. 1991; 79:428–37. [PubMed: 1751370]
7. Phillips AA, Shapira I, Willim RD, et al. A Multicenter Clinicopathologic Experience of HTLV-1 ATLL: A Retrospective 15 Year Review Reveals Little Progress. *ASH Annual Meeting Abstracts*. 2007; 110 Abstract 3569.

8. Waldmann TA, Greene WC, Sarin PS, et al. Functional and phenotypic comparison of human T cell leukemia/lymphoma virus positive adult T cell leukemia with human T cell leukemia/lymphoma virus negative Sezary leukemia, and their distinction using anti-Tac monoclonal antibody identifying the human receptor for T cell growth factor. *J Clin Invest.* 1984; 73:1711–8. [PubMed: 6327770]
9. Chen J, Petrus M, Bryant BR, et al. Autocrine/paracrine cytokine stimulation of leukemic cell proliferation in smoldering and chronic adult T cell leukemia. *Blood.* 2010; 116:5948–56. [PubMed: 20858854]
10. Waldmann TA, White JD, Goldman CK, et al. The interleukin-2 receptor: a target for monoclonal antibody treatment of human T-cell lymphotropic virus I-induced adult T-cell leukemia. *Blood.* 1993; 82:1701–12. [PubMed: 8400227]
11. Phillips KE, Herring B, Wilson LA, et al. IL-2R α -directed monoclonal antibodies provide effective therapy in a murine model of adult T-cell leukemia by a mechanism other than blockade of IL-2/IL-2R α interaction. *Cancer Research.* 2000; 60:6977–84. [PubMed: 11156399]
12. Queen C, Schneider WP, Selick HE, et al. A humanized antibody that binds to the interleukin 2 receptor. *Proc Natl Acad Sci USA.* 1989; 86:10029–33. [PubMed: 2513570]
13. Junghans RP, Waldmann TA, Landolfi NF, et al. Anti-Tac-H, a humanized antibody to the interleukin-2 receptor with new features for immunotherapy in malignant and immune disorders. *Cancer Res.* 1990; 50:1495–02. [PubMed: 2406013]
14. Vincenti F, Kirkman R, Light S, et al. Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab Triple Therapy Study Group. *N Engl J Med.* 1998; 338:161–5.
15. Nashan B, Light S, Hardie IR, Lin A, Johnson JR. Daclizumab double therapy study G. reduction of acute renal allograft rejection by daclizumab. *Transplantation.* Jan.1999 67:110–5. [PubMed: 9921806]
16. Nussenblatt RB, Fortin E, Schiffman R, et al. Treatment of noninfectious intermediate and posterior uveitis with the humanized anti-Tac mAb: A phase I/II clinical trial. *Proc Natl Acad Sci USA.* 1999; 96:7462–6. [PubMed: 10377437]
17. Stevens SR, Ke MS, Parry EJ, Mark J, Cooper KD. Quantifying skin disease burden in mycosis fungoides-type cutaneous T-cell lymphomas: The Severity-Weighted Assessment Tool (SWAT). *Arch Dermatol.* 2002; 138 42.8.
18. Junghans RP, Carrasquillo JA, Waldmann TA. Impact of antigenemia on the bioactivity of infused anti-Tac antibody: Implications for dose selection in antibody immunotherapies. *Proc Natl Acad Sci USA.* 1998; 95:1752–1757. [PubMed: 9465089]
19. Koon HB, Severy P, Hagg DS, et al. Antileukemic effect of daclizumab in CD25 high-expressing leukemias and impact of tumor burden on antibody dosing. *Leuk Res.* 2006; 30:190–203. [PubMed: 16165209]
20. WHO Handbook for Reporting Results of Cancer Treatment. World Health Organization. 1979. (Accessed at http://whqlibdoc.who.int/offset/WHO_OFFSET_48.pdf on October 14, 2010)
21. Hakimi J, Chizzonite R, Luke D, et al. Reduced immunogenicity and improved pharmacokinetics of humanized anti-Tac in cynomolgus monkeys. *J Immunol.* 1991; 147:1352–9. [PubMed: 1869828]
22. Rubin LA, Kurman CC, Biddison WE, Goldman ND, Nelson DL. A monoclonal antibody 7G7/B6 binds to an epitope on the human interleukin-2 (IL-2) receptor that is distinct from that recognized by IL-2 or anti-Tac. *Hybridoma.* 1985; 4:91–102. [PubMed: 2408992]
23. Simon R. Optimal two-stage designs for phase II clinical trials. *Controlled Clinical Trials.* 1989; 10:1–10. [PubMed: 2702835]
24. R Project for Statistical Computing. (Accessed at <http://www.r-project.org> on October 4, 2010)
25. Tsukasaki K, Utsunomiya A, Fukuda H, et al. VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. *J Clin Oncol.* 2007; 25:5458–64. [PubMed: 17968021]
26. Bazarbachi A, Plumelle Y, Carlos Ramos J, et al. Meta-Analysis on the Use of Zidovudine and Interferon-Alfa in Adult T-Cell Leukemia/Lymphoma Showing Improved Survival in the Leukemic Subtypes. *J. Clin. Oncol.* 2010; 28:4177–83. [PubMed: 20585095]

27. Ekberg H, Mamelok RD, Pearson TC, et al. The challenge of achieving target drug concentrations in clinical trials: experience from the symphony study. *Transplantation*. 2009; 87:1360–1366. [PubMed: 19424036]
28. Li N, Bian H, Zhang J, Li X, Xiaoping L, Xiaoping J, Zhang Y. The Th17/T reg imbalance exists in patients with heart failure with normal ejection fraction and heart failure with reduced ejection fraction. *Clinica Chimica Acta*. 2010; 411:23–24.
29. Webster AC, Playford EG, Higgins G, Chapman JR, Craig J. Interleukin 2 receptor antagonists for kidney transplant recipients. *Cochrane Database Syst Rev*. 2004; 1 CD003897.
30. Kirchner VA, Suszynski TM, Radosevich DM, et al. Anti-CD25 antibody (daclizumab) maintenance therapy in pancreas transplantation. *Transplant Proc*. 2010; 42:2003–5. [PubMed: 20692392]
31. Chaudhuri A, Salvatierra JO, Sarwal MM. Extended daclizumab monotherapy for rejection-free survival in non-adherent adolescent recipients of renal allografts. *Pediatric Transplantation*. 2009; 13:927–32. [PubMed: 19017291]
32. Kaplan B, West P, Neeley H, et al. Use of low-dose tacrolimus, mycophenolate mofetil and maintenance IL-2 receptor blockade in an islet transplant recipient. *Clinical Transplantation*. 2008; 22:250–3. [PubMed: 18339148]
33. Sarwal MM, Vidhun JR, Alexander SR, Satterwhite T, Millan M, Salvatierra OJ. Continued superior outcomes with modification and lengthened follow-up of a steroid-avoidance pilot with extended daclizumab induction in pediatric renal transplantation. *Transplantation*. 2003; 76:1331–9. [PubMed: 14627912]
34. Kukita T, Arima N, Matsushita K, et al. Autocrine and/or paracrine growth of adult T-cell leukaemia tumour cells by interleukin 15. *Br J Haematol*. 2002; 119:467–74. [PubMed: 12406087]
35. Azimi N, Nagai M, Jacobson S, Waldmann TA. IL-15 plays a major role in the persistence of tax-specific CD8 cells in HAM/TSP patients. *Proc Natl Acad Sci USA*. 2001; 98:14559–14564. [PubMed: 11717409]
36. Chen J, Petrus M, Bryant BR, et al. Induction of the IL-9 gene by HTLV-I Tax stimulates the spontaneous proliferation of primary adult T-cell leukemia cells by a paracrine mechanism. *Blood*. 2008; 111:5163–72. [PubMed: 18339896]
37. Ju W, Zhang M, Jiang JK, et al. CP-690,550, a therapeutic agent, inhibits cytokine-mediated Jak3 activation and proliferation of T cells from patients with ATL and HAM/TSP. *Blood*. 2011; 117:1938–46. [PubMed: 21106989]
38. Onimaru Y, Tsukaski K, Murata K, et al. Autocrine and/or paracrine growth of aggressive ATLL cells caused by HGF and c-Met. *Int J Oncol*. 2008; 33:697–703. [PubMed: 18813782]
39. Wynn D, Kaufman M, Montalban X, et al. Daclizumab in active relapsing multiple sclerosis (CHOICE study): a phase 2, randomised, double-blind, placebo-controlled, add-on trial with interferon beta. *The Lancet Neurol*. 2010; 9:381–390.
40. Bielekova B, Richert N, Howard T, et al. Humanized anti-CD25 (daclizumab) inhibits disease activity in multiple sclerosis patients failing to respond to interferon beta. *Proc Natl Acad Sci USA*. 2004; 101:8705–8708. [PubMed: 15161974]
41. Bielekova B, Catalfamo M, Reichert-Scrivner S, et al. Regulatory CD56^{bright} natural killer cells mediate immunomodulatory effects of IL-2R α -targeted therapy (daclizumab) in multiple sclerosis. *Proc Natl Acad Sci USA*. 2006; 103:5941–5946. [PubMed: 16585503]
42. Bielekova B. Daclizumab therapy for multiple sclerosis. *Neurotherapeutics*. 2013; 10:55–67. [PubMed: 23055048]
43. Wuest SC, Edwan JH, Martin JF, et al. A role for interleukin-2 trans-presentation in dendritic cell-mediated T cell activation in humans, as revealed by daclizumab therapy. *Nat Med*. 2011; 17:604–U125. [PubMed: 21532597]
44. Waldmann TA, White JD, Carrasquillo JA, et al. Radioimmunotherapy of interleukin-2R α -expressing adult T-cell leukemia with Yttrium-90-labeled anti-Tac. *Blood*. 1995; 86:4063–75. [PubMed: 7492762]
45. Kreitman RJ, Wilson WH, White JD, et al. Phase I trial of recombinant immunotoxin anti-Tac (Fv)-PE38 (LMB-2) in patients with hematologic malignancies. *J Clin Oncol*. 2000; 18:1622–36. [PubMed: 10764422]

Highlights

- The interleukin-2 receptor alpha chain (CD25) is overexpressed by ATL leukemia/lymphoma cells.
- IL-2R alpha (CD25) represents an attractive target for monoclonal antibody treatment of adult T-cell leukemia.
- Daclizumab (anti-CD25) is a humanized monoclonal antibody that blocks IL-2 binding to IL-2R alpha.
- 8 mg/kg of daclizumab was required to achieve 95% saturation of CD25 targets on ATL cells in lymph nodes.
- Up to 8 mg/kg of daclizumab administered every 3 weeks was well tolerated.
- Partial responses were observed in patients with chronic and smoldering ATL.
- The pharmacokinetics/pharmacodynamics of daclizumab provides a rationale for high-dose treatment in lymphoid malignancies and autoimmune diseases (e.g. multiple sclerosis).

Figure 1. Mean concentration time curves are plotted at each dose level for intravenous daclizumab

Daclizumab demonstrated nonlinear pharmacokinetics, typical of monoclonal antibodies, through an increased dose-normalized exposure. This may be due to saturation of an elimination pathway, a CD25(+) tumor “antigen sink,” that involves the CD25 IL-2R α receptor.

Fig 2

Figure 2-b

Figure 2-c

Figure 2-a.

Clearance (mL/hr) vs soluble TAC (units/mL); Figure 2-b. Clearance (mL/hr) vs body weight (kg) and Figure 2-c. Volume of distribution V(L) vs soluble TAC (unit (U/mL).

Figure 3. Flow cytometry was used to define saturation of CD25 by daclizumab on circulating ATL cells

The circulating ATL leukemic cells were defined by the CD25 high (upper panels), CD3 dim population using daclizumab anti-CD25 PE (upper panels) identifying the daclizumab target epitope (upper panels) as well as with the 7G7 monoclonal antibody that binds to a distinct non-competing epitope of the IL-2R α peptide (lower panels). In the patients shown there was 95% saturation defined by loss of high CD25 binding with PE daclizumab along with retained 7G7 binding achieved 24 hours after the initial dose of daclizumab that was maintained for over 2 weeks after the first dose, and for 3 weeks after the second daclizumab dose.

Figure 4. Definition of adverse events in the phase II study with 8 mg/kg of daclizumab
The non-laboratory and laboratory adverse events as shown in panels A and B respectively.

Figure 5. Best response of circulating CD4+ CD25+ (7G7+) ATL cells in patients treated with 8 mg/kg of daclizumab

The best response in circulating CD4+/CD25+ leukemic cells of each patient is shown in a waterfall plot.

Figure 6. Outcome for all patients treated with daclizumab at 8 mg/kg

(A) Progression-free survival for all patients (N=23); (B) Overall survival of all patients (N=23); (C) Progression-free survival for acute/lymphoma patients (N=11); (D) Overall survival acute/lymphoma (N=11); (E) Progression-free survival for chronic/smoldering patients (N=12); (F) Overall survival chronic/smoldering (N=12). Dashed lines represent 95% confidence intervals.

Table 1

Phase II Patient Characteristics

Characteristic		Number (percent)
Total patients Phase II [N]		20
Median age in years [range]		45[14-79]
Sex		
	Male	9(45)
	Female	11(55)
Ethnicity		
	African-Caribbean	15(75)
	Hispanic	3(15)
	Caucasian	1(5)
	West African	1(5)
Clinical Subtype		
	Acute	4(20)
	Lymphomatous	4(20)
	Chronic	3(15)
	Smoldering	9(45)
Median PS (Karnofsky) [range]		90[90-100]
	100	6(30)
	90	12(60)
	80	2(10)
Median prior therapies [range]		1(0-3)
	0	6(30)
	1	8(40)
	2	4(20)
	3	2(40)
Prior systemic chemotherapy		7(35)

Table 2

Daclizumab Pharmacokinetics from Non-compartmental Analysis

	4 mg/kg (n=5)	6 mg/kg (n=3)	8 mg/kg (n=3)
C _{max} ^{1*} (g/mL)	76.1 ± 23.5	88.6 ± 8.0	185.3 ± 67.1
AUC _{INF} [*] (h * g/mL)	7196 ± 4365	13299 ± 3718	24169 ± 10553
t _{1/2} [*] (day)	2.98 ± 2.13	7.75 ± 2.71	4.79 ± 1.87
V _{ss} [□] (L)	3.87 ± 1.16	5.97 ± 2.13	4.69 ± 1.97
CL [□] (mL/hr)	49.83 ± 19.45	26.70 ± 4.07	32.21 ± 21.63

¹ Abbreviations: C_{MAX} (maximum plasma concentration), AUC_{INF} (area under the plasma concentration vs time curve extrapolated to time infinity), T_{1/2} (half-life), V_{SS} (volume of distribution at steady rate), CL (total body clearance)

* Arithmetic Mean +/- SD

□ Geometric mean +/- SD