Phase IB Clinical Trial of the Oligosaccharide Processing Inhibitor Swainsonine in Patients with Advanced Malignancies

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ABSTRACT

The indolizidine alkaloid swainsonine, a potent inhibitor of Golgi α-mannosidase II, has been shown to reduce tumor cell metastasis, enhance cellular immune responses, and reduce solid tumor growth in mice. In our previous Phase I study, swainsonine administered by 5-day continuous infusion inhibited α-phytohemagglutinin-reactive peripheral blood lymphocytes. Significant toxicities included edema and elevated serum aspartate aminotransferase (AST). One patient with head and neck cancer had objective (>50%) tumor remission. Two patients showed symptomatic improvement. The objectives of this Phase IB trial were to examine the pharmacokinetics, toxicities, and biochemical effects of bi-weekly oral swainsonine at escalating dose levels (50–600 μg/kg) in 16 patients with advanced malignancies and 2 HIV-positive patients unsuitable for conventional therapy.

Eastern Cooperative Oncology Group performance status was ≤2. The maximum tolerated dose was defined as 300 μg/kg/day due primarily to serum AST abnormalities and dyspnea. Other adverse events present in >20% of patients included increase in serum AST (all patients), fatigue (n = 9), anorexia (n = 6), dyspnea (n = 6), and abdominal pain (n = 4). Inhibition of Golgi α-mannosidase II occurred in a dose-dependent manner. Examination of immunological parameters revealed a transient decrease in CD25 peripheral blood lymphocytes and, in seven of eight patients, an increase in CD4+/CD8+ ratios at 2 weeks. Serum drug levels peaked 3–4 h following a single oral dose in most patients and were proportional to dose at levels ≥150 μg/kg. We conclude that oral swainsonine is tolerated by chronic intermittent administration at doses up to 150 μg/kg/day. Adverse events considered drug related were similar to those observed in the infusional study but with fatigue and neurological effects also noted. Investigations of alternative dosing schedules with low starting doses are suggested for further clinical testing.

INTRODUCTION

Cell surface carbohydrate structures participate in cell-cell and cell-substratum interactions affecting lymphocyte trafficking, immune cell stimulation and function, embryogenesis, and cancer metastasis (1–3). Structural diversity of carbohydrates found on secreted and transmembrane glycoproteins are a result of tissue-specific regulation of Golgi enzymes required in their biosynthesis (Refs. 4–6; Fig. 1). Cancer cells commonly show increased GlcNAc-transferase V activity and the resulting β1–6 GlcNAc-branched N-linked carbohydrate structures (7–10). We have shown that the degree of β1–6GlcNAc-branched in rodent tumor models (11, 12) and in human breast and colon carcinomas (13, 14) correlates with disease progression. Furthermore, inhibitors of the N-linked carbohydrate processing pathway have been shown to attenuate both metastasis and tumor growth in animal models (15–18).

Swainsonine, or 8β-indolizidine-1a,2a,8b-triol, is an indolizidine alkaloid first isolated from the Australian plant Swainsona canescens (19), and later from North American plants of the genera Astragalus and Oxystropis (20). Swainsonine inhibits Golgi α-mannosidase II and consequently the carbohydrate processing pathway prior to the initiation of the β1–6 GlcNAc-linked branch (Fig. 1). Tumor cells cultured in the presence of swainsonine show enhanced substratum adhesion and loss of invasiveness into extracellular matrix, as well as loss of organ colonization potential when injected i.v. into mice (16, 21, 22). Given to mice p.o. (15, 23–25), by i.p. injection (26), and by systemic infusion (27), swainsonine has been shown to inhibit solid tumor growth of murine lymphoma as well as human melanoma and breast and colon carcinoma xenografts. In addition to the effects on tumor cell invasion, swainsonine alleviates tumor-induced and chemically induced immune suppression (26, 28, 29) and stimulates both natural killer cell (30) and macrophage (30, 31) activity in mice. The addition of swainsonine to human lymphocyte cultures has been shown to augment lymphocyte-activated killer cell-induced killing of human colon carcinoma cells (32). These observations strongly suggest that swainsonine may be useful for the treatment of human malignancy.

1 The abbreviations used are: GlcNAc, N-acetylglucosamine; MTD, maximally tolerated dose; DLT, dose-limiting toxicity; AST, aspartate aminotransferase; PHA, phytohemagglutinin; PBL, peripheral blood lymphocyte; FCM, flow cytometry; FITC, fluorescein isothiocyanate.

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Two Phase IB studies of swainsonine in patients with advanced malignancies have now been completed. In our first study (33), swainsonine was administered to 19 patients at repeated, 28-day intervals by continuous iv. infusion over 5 days in escalating doses from 50–550 μg/kg/day. The MTD and the recommended starting dose (MTD-1 level) were 550 and 450 μg/kg/day, respectively. Increases in serum AST levels were dose limiting. Other adverse events included dyspnea, edema, fatigue, anorexia, mild alanine aminotransferase elevation, a rise in serum amylase, and decreased serum retinol levels. One patient died from an acute respiratory distress syndrome that was possibly treatment related, although postmortem findings indicated significant tumor within the lungs and liver, as well as bilateral pneumonia. One patient with head and neck cancer showed >50% shrinkage of tumor mass. A marked decrease in t-PHA binding to PBLs was detected after the 5-day course of treatment, consistent with inhibition of the target enzyme Golgi α-mannosidase II. Oligomannosides accumulated in patient urine, reflecting inhibition of tissue lysosomal α-mannosidases, and reached steady state at 3 days, only 1 day after serum drug steady state.

Here we describe our second clinical study that examined the use of swainsonine given by oral administration in twice weekly doses of 50–600 μg/kg. In animal studies, inhibition of solid tumor growth and immune stimulation has been demonstrated when swainsonine was given p.o. either by chronic or intermittent schedules (24). In the present Phase IB study of oral swainsonine, we have measured qualitative and quantitative toxicities, serum drug levels, and markers of α-mannosidase inhibition and lymphocyte distribution.

**PATIENTS AND METHODS**

Eighteen patients were enrolled in the chronic oral study between July 1993 and February 1994. The study was approved by the University of Toronto and The Toronto Hospital Ethics Committee. Patients ≥18 years with a life expectancy of at least 3 months, Eastern Cooperative Oncology Group performance status 0–2, and histologically confirmed diagnosis of metastatic cancer refractory to standard effective therapy, or for which no conventional therapy exists, were eligible for enrollment on study. HIV-positive patients unsuitable for conventional therapy were also eligible. All patients were required to have completed pre-study chemotherapy or radiotherapy at least 3 weeks prior to enrollment and have signed an informed consent. Exclusion criteria included inadequate organ function (hepatic: bilirubin >20 μmol/liter or AST ≥2 times normal; renal: creatinine >130 or creatinine clearance ≤1 ml/s), central nervous system metastases, or prior exposure to experimental anticancer agents. Screening prior to enrollment included: signed informed consent; history and physical exam; assessment of Eastern Cooperative Oncology Group performance status; chest X-ray; routine hematology, biochemistry, urinalysis, and electrocardiogram; blood and urine collection for baseline PBL marker analysis by FCM; plasma drug levels; and urine oligomannosides. Baseline symptoms were recorded.

During the chronic oral dose schedule patients were examined every 2 weeks, at which time any observed toxicities were recorded and physical examinations were performed. Routine hematology, biochemistry, plasma drug level, and urine oligomannoside analysis were done at each visit. FCM analysis was conducted before treatment initiation and at 2 and 4 weeks of biweekly swainsonine treatment. All samples were collected prior to swainsonine administration that day. Some patients did not return for follow-up visits at exact 2-week interval or did not provide blood and/or urine samples. Only those patients with complete blood sample sets (baseline and 2 and 4 weeks on biweekly swainsonine treatments) were included in our FCM data analysis. Blood and urine was not collected from HIV-positive individuals. Chest X-rays and EKGs were repeated as determined appropriate by the investigator.

**Swainsonine Administration.** Swainsonine was synthesized by Toronto Research Chemicals. Purity was confirmed by proton nuclear magnetic resonance spectroscopic analysis to be greater than 98%. Appropriate concentrations of swainsonine were prepared by the Pharmacy Department of the Toronto Hospital by reconstituting the drug in 30 ml of sterile water in 30-ml/unit dose polypropylene vials. Reconstituted swainsonine is a colorless and tasteless preparation. Chronic oral scheduling consisted of twice weekly doses (Mondays and Thursdays)
administered before the evening meal. The chronic oral dose levels were 50, 150, 300, and 600 µg/kg on each treatment day.

Pharmacokinetics. Swainsonine was prepared for treatment at single oral bolus administration in concentrations of 50, 150, 250, 500, and 1000 µg/kg. Blood samples were drawn from a saline lock inserted into the patient's arm vein at t = 0, 0.33, 0.66, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, and 6.0 h following swainsonine administration. Serum swainsonine levels were fit to multieponential equations by nonlinear regression. The relatively short sampling interval allowed only two pharmacokinetic parameters, maximum swainsonine concentration (Cmax) and time to maximum (Tmax), to be calculated.

Dose Escalation. Three patients were treated at each dose level. Intrapatient dose escalation was not allowed. Dose escalation occurred until the MTD was reached. MTD was defined as that dose level at which at least two of three patients experienced DLT. Once all three patients in a cohort had been followed for 1 month (from their first dose of swainsonine) and evidence of DLT was seen either in one or fewer of these patients, new patients were treated at the next higher dose level. DLT was defined as any grade 3 or 4 nonhematological toxicity according to standard National Cancer Institute of Canada toxicity criteria (used with the permission of the National Cancer Institute of Canada). When MTD was defined, three additional patients were treated one dose level lower to ensure that MTD had not been exceeded.

Serum Drug Levels. The method used for extraction, acetylation, and measurement of serum swainsonine has been slightly modified from our protocol published previously (34). The dry samples were acetylated by the addition of 18 mg of 4-dimethylaminopyridine, 18 mg of sodium acetate, 1 ml of acetonitrile, and 500 µl of acetic anhydride. The sealed reaction tubes were heated overnight in a 70°C water bath. The mixture of reaction products was partitioned by adding 1 ml of chloroform, 1 ml of water, and mixing. The lower chloroform layer was transferred by Pasteur pipette to a clean tube. Two further additions of 1 ml of chloroform were made to the mixture of products and mixed; then the lower chloroform layer was pooled for each sample. The chloroform solution was washed with 1 ml of water to further remove polar products and unreacted reagents from the solution. The lower chloroform layer was passed through a solid phase extraction cartridge of 500 mg of basic alumina to clean the sample; then it was concentrated under nitrogen at 60°C. The samples were quantitatively transferred to GC vials with micro inserts, evaporated at ambient temperature, and then rediluted in 100 µl of chloroform. If problems were encountered in the column clean-up step and a strongly yellow colored solution was observed, the samples were diluted to 130 µl to improve GC separation. The sample GC injection volume was 1 µl.

The samples were analyzed using a Hewlett Packard G 1800A GCD gas chromatograph-electron impact quadrupole mass spectrometer. A 30-m long, 0.25 mm internal diameter, 0.25-mm film thickness HP-5 MS column was used for the GC separation. The inlet temperature was 225°C, the detector temperature was 280°C, and the initial column temperature was 110°C. The temperature program was: 110°C for 2 min, then increase at a rate of 10°C/min to 190°C, hold 12 min, increase at 5°C/min to 220°C, hold for 2 min and then increase at 20°C/min to 260°C, and then hold for 6 min. Helium was used as the carrier gas, with a flow rate of 1.0 ml/min. The mass spectrometer detection parameters for selected ion mode were m/z 120 and 137 (after 6 min solvent delay) to 17.0 min. Ions m/z 115 and 157 were also monitored between 17.0 and 18.0 min and exclusively monitored after 18.0 min to the end of the run. The acetylated swainsonine in standards and samples was identified at approximately 16.3 min. Identity was confirmed by ratios of the m/z 120:137 ions and checking the elution profiles for each ion. Similarly, the primary internal standard, methyl α-d-mannopyranoside, was detected at 18.4 min, using the m/z 115, m/z 157 ion signals and ratios. The secondary internal standard, methyl β-d-galactopyranoside, was detected at 18.8 min with ions m/z 115 and 157.

Sample and standard concentrations were determined by integrating the combined signal of the two ions used for detection. A five point external standard curve, with internal standards, was included for every 25 samples. Chloroform blanks were included every 10 samples to ensure that column background was negligible. The primary internal standard (methyl α-d-mannopyranoside) was used as a recovery standard. The secondary internal standard was used to confirm the primary internal standard (ratios of signals).

FCM Analysis of PBLs. PBLs were prepared and analyzed for CD3, CD4, CD8, CD14, CD16, CD25, CD57, and HLA-DR on a Coulter Profile (Coulter). Peripheral blood was collected in heparin-containing tubes, transported at room temperature, and analyzed within 48 h. Monoclonal antibodies conjugated with either FITC or phycoerythrin were purchased from Becton Dickinson or Coulter. FITC-labeled 1-PHA was from E.Y. Labs. Aliquots of 100 µl of whole blood were incubated with anti-CD antibodies or 0.1 µg/ml of FITC-labeled 1-PHA for 20 min followed by lysis of RBCs, washing by centrifugation, and fixation of the cells by using the automated Q Prep system (Coulter). The lymphocyte population was gated, and the contaminating monocytes were assessed by staining with CD14, which was consistently below 1% of the gated population.

RESULTS

Twenty-two patients were enrolled in the study. Of these, 19 had a diagnosis of malignancy and 3 were HIV positive. Three patients (two with malignancy and one with HIV) completed only the pharmacokinetic portion of the study and did not proceed to biweekly swainsonine treatments. One patient never received swainsonine treatment. The characteristics of the patients treated at the four dose levels are summarized in Table 1. Median duration of biweekly swainsonine treatment was 4 weeks and ranged from 1 to 33 weeks. MTD was defined according to patient toxicities occurring with 1 month of swainsonine treatment as described in Table 2.

MTD Determination. The first three patients enrolled in each of the dose groups of 50 and 150 µg/kg twice weekly did not experience any DLT within the first month of treatment. One of the three patients in the 300 µg/kg/day dose group experienced DLT after 1 month (worsening edema). Due to inaccurate weight measurement, one patient (C. F.) received 417 µg/kg biweekly oral doses. This patient was included in the 300 µg/kg
of treatment. Thus, of the remaining six patients at this dose level, one experienced DLT. This confirmed 300 µg/kg/day as the MTD. The patient experiencing DLT had lung cancer and preexisting moderate dyspnea, which worsened to grade 3 dyspnea after approximately 1 month of therapy. Study drug was discontinued after 6 weeks because of this adverse event.

Treatment-related Adverse Events and Patient Outcome. An increase in serum AST during chronic oral swainsonine and a decrease in serum AST following cessation of treatment was observed in all patients as shown for dose level 150 µg/kg/day in Fig. 2. For all patients, the median AST on treatment rose to 85 ± 46 units/liter from an average baseline of 45 ± 35 units/liter (P = 0.0001). The effect was dose dependent (P < 0.0001). The magnitude of increase in serum AST appeared to correlate with baseline liver function. Notably, grade 3 AST abnormalities only occurred in those patients with pretreatment liver dysfunction. The three patients experiencing grade 3 AST abnormalities had their swainsonine dose reduced. Two patients (P. G. and R. F.) exhibited grade 3 elevation in serum bilirubin based on a single measurement taken after the first 4 weeks of swainsonine treatment. In both cases, bilirubin returned to normal within 2 weeks and stayed within the normal range for the remainder of the treatment period.

Peripheral edema attributed to disease-associated lymphatic obstruction was present in five patients at baseline. In three of these patients, edema worsened while on study (see Table 2). Six patients experienced dyspnea on study (grade 1, n = 2; grade 2, n = 3; grade 4, n = 1). All of those with dyspnea more than grade 1 on study had lungs involved with tumor. As indicated above, one patient (P. S.) with fibrohistiocytoma metastatic to lung exhibited pulmonary edema, probably attributable to swainsonine, which was reversible when treatment was discontinued. In two other patients (A. Z. and I. Y.), the relationship between worsening dyspnea and swainsonine treatment was unclear. The fourth patient (N. K.) developed grade 4 dyspnea while on study and died within 2 weeks of his last swainsonine treatment. Autopsy findings ruled out extensive pulmonary edema and indicated respiratory failure due to disseminated adenocarcinoma, thereby suggesting the observed shortness of breath was not drug related. Significant, clear serous exudate was observed weeping from the advanced chest wall lesions of one subject (V. K.) 1.5 weeks after initiation of swainsonine treatment.

Two patients (C. F. and R. B.) reported unilateral numbness during swainsonine treatment. In another patient (N. K.), preexisting lower limb paraesthesia worsened during treatment, and the patient developed twitching. One of these patients (C. F.) experienced a 4-day episode of dizziness, tachycardia, and unsteadiness directly related to cessation of amitriptyline. Symptoms resolved within 24 h of amitriptyline reinitiation. Follow-up brain computed tomography scan investigations were negative for R. B., but C. F. was found to have a parasagittal meningioma. Three months following study termination, C. F. was asymptomatic. R. B. was asymptomatic 2 weeks after the study.

Other adverse events occurring with a frequency of >20% (i.e., >4 patients) included fatigue (n = 9), anorexia (n = 6), dyspnea (n = 6), and abdominal pain (n = 4).

At the time of manuscript preparation, six patients were
Table 2 Drug-related toxicities occurring within 1 month of swainsonine treatment

<table>
<thead>
<tr>
<th>Dose level/ Patient</th>
<th>Disease site</th>
<th>Edema (peripheral)</th>
<th>Fatigue (grade)</th>
<th>Dyspnea</th>
<th>AST &lt; 35 units/liter (iu)</th>
<th>ALT &lt; 40 units/liter</th>
<th>Bilirubin &lt; 20 mmol/liter</th>
<th>Amylase &lt; 115 mmol/liter</th>
<th>Grade*</th>
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<tr>
<td>I: 50 μg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P.G. Lung</td>
<td>Lung</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>102</td>
<td>26</td>
<td>27</td>
<td>57</td>
<td>1.0</td>
</tr>
<tr>
<td>L.T. Colon</td>
<td>Colon</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>70</td>
<td>19</td>
<td>14</td>
<td>61</td>
<td>1.0</td>
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<tr>
<td>R.F. Renal</td>
<td>Renal</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>12</td>
<td>19</td>
<td>43</td>
<td>1.0</td>
</tr>
<tr>
<td>II: 150 μg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N.H. HIV positive</td>
<td>Lung</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>87</td>
<td>72</td>
<td>20</td>
<td>76</td>
<td>1.0</td>
</tr>
<tr>
<td>E.P. Lung</td>
<td>Lung</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>57</td>
<td>36</td>
<td>13</td>
<td>109</td>
<td>1.0</td>
</tr>
<tr>
<td>M.C. HIV positive</td>
<td>Lung</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>126</td>
<td>76</td>
<td>14</td>
<td>69</td>
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<tr>
<td>A.Z. Lung</td>
<td>Lung</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>101</td>
<td>34</td>
<td>16</td>
<td>109</td>
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<tr>
<td>V.K. Breast</td>
<td>Breast</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>64</td>
<td>16</td>
<td>13</td>
<td>121</td>
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<tr>
<td>A.T. Adrenal</td>
<td>Adrenal</td>
<td>0</td>
<td>0</td>
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<td>76</td>
<td>18</td>
<td>16</td>
<td>52</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>S.O. Melanoma</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>110</td>
<td>34</td>
<td>19</td>
<td>120</td>
<td>2.0</td>
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<tr>
<td>C.F. Hypopharyngeal</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>84</td>
<td>16</td>
<td>17</td>
<td>29</td>
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<tr>
<td>T.Y. Hodgkin’s</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>76</td>
<td>27</td>
<td>10</td>
<td>152</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>R.B. Fibrosarcoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>224</td>
<td>85</td>
<td>18</td>
<td>208</td>
<td>3.1</td>
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<tr>
<td>P.S. Fibrohistiocytoma</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>99</td>
<td>67</td>
<td>13</td>
<td>ND</td>
<td>2.1</td>
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<tr>
<td>IV: 600 μg/kg</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N.K. Pancreatic</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>292</td>
<td>63</td>
<td>16</td>
<td>ND</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>E.S. Lung</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>256</td>
<td>86</td>
<td>23</td>
<td>89</td>
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</tbody>
</table>

Two patients experienced grade 2 nausea/vomiting. With the exception of M. C. (Kaposi’s sarcoma), HIV patients enrolled in the study may not have had confirmed cancer. No blood or urine samples were collected from these patients.

* Pertaining to National Cancer Institute of Canada grading of peak test result indicated in the preceding four columns as follows: AST, ≤ 2.5 X N = grade 1, 2.6–5.0 X N = grade 2, 5.1–20 X N = grade 3; alanine aminotransferase, ≤ 2.5 X N = grade 1, 2.6–5.0 X N = grade 2, 5.1–20 X N = grade 3; bilirubin, < 1.5 X N = grade 2, 1.5–3.0 X N = grade 3; amylase, < 1.5 X N = grade 1, 1.5–20 X N = grade 2, 2.1–5.0 X N = grade 3.
* Patients with abnormal liver function tests prior to initiation of swainsonine therapy.
* Patients experiencing neurological side effects (numbness in limbs).
* Shortness of breath present pre-study did not change within the first 4 weeks of swainsonine initiation.
* Patients who had dose reduced due to DLT.
* Increased to grade 4 in 6 weeks of therapy.

alive with a median time from final swainsonine treatment of 83 weeks. Twelve patients had died with a median time from last swainsonine treatment of 14 weeks. Ten of 18 patients were withdrawn from chronic oral swainsonine treatment because of disease progression. Eight patients discontinued treatment because of adverse events including: fatigue (n = 3); dyspnea (n = 3); fatigue, dyspnea, and anorexia (n = 1); and headache, numbness, and parasthesia (n = 1). Two patients discontinued treatment for other reasons. Disease response was not an end point of this study. In those patients in whom disease was evaluable, however, there were no obvious objective responders.

**Pharmacokinetics.** Sixteen patients were enrolled in this part of the study at the following doses: 50 μg/kg (n = 4); 150 μg/kg (n = 1); 250 μg/kg (n = 5); 500 μg/kg (n = 2); 700 μg/kg (n = 1); and 1000 μg/kg (n = 2). Plasma level data were available on 12 of these patients. Thirteen patients entered the chronic oral study with 5 additional patients enrolled for a total of 18 patients. Analysis of swainsonine levels following a single oral bolus found that 7 of 12 patients exhibited rapid absorption of swainsonine with peak plasma levels attained within 3 h of dosing. Data analysis was limited to C_max and T_max because swainsonine blood levels did not return to zero within the sampling period. Table 3 summarizes these two pharmacokinetic parameters by dose group and patient. The mean peak blood levels ranged from 0.3 to 1.62 μg/ml across the dose groups. In the upper three dose groups (doses ≥ 150 μg/kg), C_max can be considered proportional to dose as indicated by the approximately constant dose-adjusted C_max [within the range of 1.3–1.6 (μg/ml)1/4(μg/kg)]. At the lowest dose (50 μg/kg), the dose adjusted maximum concentration was considerably higher, with a mean value for the group of 6.1 (μg/ml)1/4(μg/kg). A single patient (S. O.) in the upper dose groups had a higher dose-adjusted C_max than the three patients in the 50-μg/kg group. Mean T_max varied in the range of 160–240 min with no obvious dose-relationship.

**Inhibition of Golgi and Lysosomal α-Mannosidas.** L-PHA lectin binds to β1–6GlcNAc-branched complex-type oligosaccharides (35) and, therefore, can be used to monitor inhibition of the Golgi oligosaccharide processing pathway by swainsonine (Fig. 1). The glycoproteins synthesized by PBLs during swainsonine treatment are expected to bear hybrid-type oligosaccharides rather than the L-PHA-reactive complex-type structures. In this study, L-PHA binding to PBLs collected pretreatment and just prior to the month 1 swainsonine dose revealed a dose-dependent decrease in β1–6GlcNAc-branched complex-type oligosaccharides (P = 0.0441; Fig. 3). A transitory increase in L-PHA binding was observed in 5 of 10 patients at 2 weeks of treatment, notably in patients treated with doses ≤ 300 μg/kg.

Swainsonine inhibits both Golgi α-mannosidase II and lysosomal α-mannosidases with a similar K_i of approximately 100 nm. Blocking the latter enzyme causes accumulation of oligomannosides in tissues and body fluids (36, 37). Fluorophore-assisted carbohydrate electrophoresis was used to quan-
tify reducing oligosaccharides in urine collected at 2-week intervals from five patients as described previously (33). Urine oligomannosides were detected after 2 weeks on swainsonine treatment, and levels increased with continued treatment to 4 weeks (data not shown). This suggests that plasma drug levels attained during the twice weekly dosing schedule are not associated with rapid saturation of tissue α-mannosidases as observed previously in the i.v. infusion trial, where urine oligomannosides reached steady-state concentrations 72 h after starting swainsonine infusion at doses of 150 μg/kg/day or greater (33).

Analysis of PBL Cell Surface Markers. Ten of the 18 patients who received chronic oral swainsonine had lymphocyte counts below normal at baseline, which may reflect either underlying advanced malignancy or extensive prior anticancer treatment characteristic of this study population. In studies in animals, swainsonine has been shown previously to augment lymphocyte proliferation (29). No significant changes were found for hematological parameters in this study. However, a trend toward an increase in average granulocyte values following swainsonine treatment was noted. Mean values increased 33% over baseline. Six individual patient profiles showed granulocyte increases from 22 to 183% over baseline values in the 6-month period (range, 2–21 weeks) following first exposure to swainsonine.

Swainsonine administered to mice has been shown to increase natural killer cell activity (23) and class II antigen 1a expression (30, 31) in hematopoietic cell populations. PBLs collected from eight patients (pretreatment and prior to daily swainsonine dose on study) were, therefore, examined for changes in CD3- CD4-, CD8-, HLA-DR-, CD25-, CD16-, and CD14-positive cells. The total number of CD3+ T-cells expressing CD25 (interleukin 2 receptor antigen) was found to decrease with 2 weeks of oral swainsonine treatment in all patients tested (Fig. 4). After 1 month of treatment, the percentage of T cells expressing CD25 had increased from the levels observed at 2 weeks. In two of eight patients, the percentage measured at 1 month exceeded pretreatment levels. The ratio of CD4+ :CD8+ (i.e., helper:T killer/apressor cell) was demonstrated to increase with 2 weeks of chronic oral swainsonine treatment in seven of eight patients tested (Fig. 5). No specific trends were noted in CD3, HLA-DR, CD16, or CD14 expression (data not shown).

DISCUSSION
Upon completion of this second study, adverse events attributable to swainsonine given by continuous 5-day i.v. infusion (as in our previous study; Ref. 33) or chronic twice weekly oral dosing (described in this study) can be compared as follows. Serum AST elevation occurred in both studies but was dose dependent in the chronic oral schedule only. Pulmonary edema (with associated dyspnea) and peripheral edema occurred in both studies, and neurological symptoms were not observed in the i.v. study but did occur in three patients receiving the oral schedule. From this comparison, it appears that serum AST elevation, edema, and dyspnea occur with even short-term exposure to swainsonine, whereas if the neurological side effects are due to swainsonine, they may be related to repeated dosing over a prolonged period.
Table 3  Pharmacokinetics: single oral dose of swainsonine

<table>
<thead>
<tr>
<th>Dose group (µg/kg)</th>
<th>Patient</th>
<th>Dose µg/kg</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; µg/ml</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;/Dose x 1000</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (min)</th>
</tr>
</thead>
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<tr>
<td>50</td>
<td>P. G.</td>
<td>50</td>
<td>0.50</td>
<td>10.08</td>
<td>75</td>
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<tr>
<td></td>
<td>I. T.</td>
<td>50</td>
<td>0.15</td>
<td>3.00</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>R. F.</td>
<td>50</td>
<td>0.26</td>
<td>5.20</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>0.30</td>
<td>6.09</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>S. D.</td>
<td></td>
<td>0.18</td>
<td>3.62</td>
<td>113</td>
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<tr>
<td>150-250</td>
<td>I. B.</td>
<td>150</td>
<td>0.25</td>
<td>1.67</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>I. G.</td>
<td>250</td>
<td>0.31</td>
<td>1.24</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>A. Z.</td>
<td>250</td>
<td>0.28</td>
<td>1.12</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>0.28</td>
<td>1.34</td>
<td>163</td>
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<tr>
<td></td>
<td>S. D.</td>
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<td>0.03</td>
<td>0.29</td>
<td>15</td>
</tr>
<tr>
<td>500-700</td>
<td>S. O.</td>
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<td>1.60</td>
<td>3.20</td>
<td>245</td>
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<tr>
<td></td>
<td>T. Y.</td>
<td>500</td>
<td>0.46</td>
<td>0.92</td>
<td>&gt;300</td>
</tr>
<tr>
<td></td>
<td>C. F.</td>
<td>700</td>
<td>0.40</td>
<td>0.57</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>0.82</td>
<td>1.56</td>
<td>240</td>
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<tr>
<td></td>
<td>S. D.</td>
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<td>1.43</td>
<td>63</td>
</tr>
<tr>
<td>1000</td>
<td>R. B.</td>
<td>1000</td>
<td>1.30</td>
<td>1.30</td>
<td>&gt;300</td>
</tr>
<tr>
<td></td>
<td>N. K.</td>
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<td>170</td>
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<tr>
<td></td>
<td>E. S.</td>
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<td>1.75</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>1.62</td>
<td>1.62</td>
<td>223</td>
</tr>
<tr>
<td></td>
<td>S. D.</td>
<td></td>
<td>0.27</td>
<td>0.27</td>
<td>68</td>
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</table>

Pharmacokinetic data generated from serum drug levels measured at times following a single oral dose of swainsonine as indicated. The serum drug levels were measured by gas chromatography-mass spectrometry as described in "Materials and Methods." C<sub>max</sub>/dose is the dose-adjusted C<sub>max</sub>. * Estimates based on curve fitting.

Fig. 3  1-PHA lectin binding to total PBLs of patients pre-swainsonine treatment (Pre-Tx) and at 2 and 4 weeks of treatment. 1-PHA binding was measured by FCM as mean fluorescence intensity. Results are graphed as percentage of change from pretreatment levels. Right, patients' initials.

Possible mechanisms responsible for swainsonine-induced toxicities should be considered. Elevated serum AST levels may be related to swainsonine-induced hepatocyte damage. Increases in serum GGT (data not reported) observed concomitant with elevated AST levels in our i.v. study support this explanation. Furthermore, aminotransferase abnormalities were most severe in patients with pretreatment liver dysfunction and/or in those with liver metastases. It has been shown, however, that swainsonine is not cytotoxic to cells in tissue culture (15, 16). Alternatively, elevated serum AST levels may result from alterations in enzyme half-life and clearance caused by swainsonine treatment. Endothelial cells, liver hepatocytes, and macrophages bind serum glycoproteins via carbohydrate-specific binding receptors, thereby regulating glycoprotein longevity in the circulation (38). It has been shown that clearance via the mannose receptor is slower in swainsonine-treated animals (39) and that slowed clearance ofmannose-terminating enzymes is reversible with discontinuation of swainsonine treatment (40). It is possible that the increase in circulating mannose-terminating glycoproteins due to α-mannosidase II inhibition by swainsonine (Fig. 1) results in saturation of endocytic and lysosomal pathways, causing slower turnover of these enzymes.
It has been suggested previously that swainsonine may cause a capillary leak syndrome (33). Swainsonine has been shown to increase lymphocyte sensitivity to interleukin 2 and other cytokines (32, 41). A swainsonine-associated capillary leak syndrome, comparable to that which has been documented in clinical studies using interleukin 2 to treat human malignancy (42, 43) could, therefore, be caused by augmented immune cell reactivity to local cytokines. This effect would be enhanced in areas of increased immune cell recruitment and activity such as tumor-involved tissues. This mechanism could account for both peripheral and pulmonary edema (causing dyspnea) occurring with swainsonine and is indicated by the presentation of dyspnea more than grade 1 only in those patients with lung involved with tumor. If this hypothesis is correct, we would not expect swainsonine to cause either pulmonary edema or significant peripheral edema in patients without underlying malignancy.

Unilateral numbness and paraesthesia were observed only with chronic oral swainsonine treatment. Neurological symp-
toms including lethargy and feed refusal are known to occur in grazing herds consuming diets rich in natural sources of swainsonine and other alkaloids (20, 44). Inhibition of lysosomal α1–3 and α1–6 mannosidase by swainsonine is known to result in accumulation of oligomannosides in tissues and body fluids (36, 37). In our clinical studies, significant urinary oligomannoside levels were detected after i.v. and oral administration of swainsonine. Studies in rodents (45) and primates⁶ have shown that oligomannoside storage can occur in the brain in the absence of neurological symptoms. Further studies are required to clarify the clinical significance of oligomannoside tissue storage.

The mechanisms responsible for swainsonine-related fatigue observed are not apparent. The lower incidence of fatigue reported in our i.v. trial may be explained by the fact that study participants were predominantly in bed during treatment. This symptom may represent neurological toxicity as discussed above: however, additional studies are required to determine its cause.

A number of the biological effects of swainsonine observed in preclinical studies were confirmed in patients receiving swainsonine treatment. Decreased T-PLA binding reflecting Golgi α-mannosidase inhibition was documented in both clinical trials. A dose-dependent effect was observed in our oral study, whereas all doses ≥150 μg/kg/day maximally inhibited the enzyme in our i.v. trial. Reduced enzyme inhibition observed with oral administration is likely related to lower maximum serum levels after twice weekly oral dosing versus continuous i.v. infusion. The mean Cmax following a single 150–250 μg/kg oral dose measured in the pharmacokinetic portion of this study was 0.28 μg/ml, whereas blood levels attained by continuous i.v. infusion with doses of 150–250 μg/kg/day were 3 mg/ml (Ref. 33). The transient increase in T-PLA binding observed at 2 weeks of oral swainsonine treatment with doses ≥300 μg/kg may indicate that low levels of swainsonine stimulate lymphocyte activation known to be accompanied by increased expression of GlcNAc-TV and T-PLA-reactive β1–6GlcNAc-branched structures (46). A number of studies of swainsonine in mice have shown that shorter, intermittent treatment periods maintain immune stimulation (18, 24, 47). Furthermore, doses producing less than a 20% decrease in normal N-linked complex type glycosylation are considered a disease-related versus drug-related phenomena, then the MTD in patients without lung involvement may well be 600 μg/kg. Further clinical studies in different patient populations are required to investigate this possibility. Greater inhibition is likely to have occurred during peak swainsonine levels reached soon after drug administration; however, the level of enzyme inhibition necessary to achieve optimal clinical efficacy is not clear. In view of these findings as well as the serum AST changes and putative cytokine-induced capillary leak syndrome noted, careful patient selection, alternative dosing schedules, low starting doses, and close toxicity monitoring will be necessary in planning further clinical trial development of this interesting agent in patients with advanced malignancies.

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⁶ J. J. Lipman, unpublished observations.
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