Phase I Trial of H65-RTA Immunoconjugate in Patients With Cutaneous T-Cell Lymphoma

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H65-RTA is an immunoconjugate that consists of the A chain of ricin (RTA), a ribosomal-inhibiting protein, coupled to a murine monoclonal antibody (H65) directed against the pan-T-cell antigen CD5. The CD5 antigen is heterogeneously expressed on cutaneous T-cell lymphoma tumor cells, but is not expressed on normal cells except lymphocytes. A phase I trial was therefore conducted in which 14 patients with cutaneous T-cell lymphoma progressive on other therapies were treated with up to three cycles of H65-RTA. The maximal tolerated dose (MTD) of H65-RTA was 0.33 mg/kg/d administered intravenously for 10 days as defined by dyspnea at rest at higher doses. Other reversible side effects included myalgia, mild hypoalbuminemia with weight gain, pedal edema, fatigue, fevers, and chills. Six patients received more than one cycle of H65-RTA without increased side effects compared with the first cycle. Pharmacokinetic analysis showed that peak serum drug levels were dose-dependent, and ranged from 1.13 to 5.66 µg/mL, with a terminal half-life ranging from 1.0 to 2.9 hours. The development of antibodies against the immunoconjugate was associated with a lower peak drug level, but not with enhanced side effects. Partial responses lasting from 3 to 8 months were documented in four patients. Three of the responding patients received more than one cycle of H65-RTA in the presence of anti-immunoconjugate antibodies. The results from this phase I trial suggest that H65-RTA is an active drug in the treatment of cutaneous T-cell lymphoma. The immunoconjugate may be safely administered repeatedly, even in the presence of anti-immunoconjugate antibodies, with responses noted. Additional studies at the MTD are needed to define the response rate in this disease.

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Materials and Methods

H65-RTA is composed of a CD5-specific murine IgG1 MoAb coupled through a chemical linker to RTA by a disulfide bond.10 The CD5 antigen is present on the cell surface of mature T lymphocytes, but not on normal nonlymphoid human tissues (XOMA Corporation, Berkeley, CA, unpublished observations). All detectable ricin B chain is removed from the RTA preparation before conjugation. The immunoconjugate used in this clinical study contained an average of 8.1% free MoAb, had an average RTA:MoAb molar ratio of 2.0, and retained an average 82.7% of the binding activity of unconjugated MoAb MOLT-4 cells (American Type Culture Collection [ATCC], Bethesda, MD), a T-cell acute lymphoblastic leukemia (ALL)-derived cell line that expresses CD5.

H65-RTA was supplied by XOMA Corporation in vials containing 3 mL of drug, with a protein concentration of 1 mg/mL. The drug was stored at 2°C to 8°C, and the appropriate dose diluted in 100 mL normal saline for administration. Immediately before the first dose of each cycle, all patients were challenged by an intravenous (IV) test dose of 100 µg and closely observed for 15 minutes for an allergic reaction. A total of 10 IV infusions of the immunoconjugate in patients diagnosed with CTCL, to determine if patients could be safely retreated, and to document antitumor activity.

Cutaneous T-Cell lymphomas (CTCL) are a heterogeneous group of T-cell malignancies that include the epidermotropic variants mycosis fungoides and Sézary syndrome. They have a variable natural history, with mycosis fungoides being relatively indolent in contrast to the other, more aggressive members of the group. In addition to a T-lymphocyte phenotype and a propensity for skin involvement, a common feature of this family of lymphomas is early systemic dissemination.12 The recognition of systemic disease has led to progressively more intensive therapy early in the course of the disease.14 Unfortunately, the majority of patients are destined to relapse, necessitating the development of additional systemic therapies.

The immunologic phenotype of CTCL is well characterized.12 Among the T-cell surface antigens expressed by this group of lymphomas is a 65-kD glycoprotein assigned to the fifth cluster designation (CD5). The expression of CD5 by CTCL, while heterogeneous, is often present in higher density than on normal lymphocytes, is identical between malignant lymphocytes infiltrating different sites, and appears to be consistent over time.78 The CD5 antigen has, therefore, been examined as a suitable target for monoclonal antibody (MoAb) therapy. Antibody binding has been shown in areas of malignant infiltration, and transient tumor regressions have been obtained using unconjugated murine MoAbs that recognize CD5.91 A radio-conjugated antibody directed towards CD5 has also shown therapeutic potential, but myelosuppression is a dose-limiting toxicity.13

H65-RTA is an immunoconjugate composed of a murine MoAb directed against the CD5 antigen conjugated to the A chain of ricin (RTA), a potent inhibitor of protein synthesis. The cytotoxic activity of RTA is dependent on the intracellular inactivation of an adenosine residue at the ribosomal binding site for elongation factor-II with resultant inhibition of protein synthesis.10,14 H65-RTA is therefore a compound that is specifically cytotoxic for cells expressing the CD5 antigen. The objectives of this study were to establish the maximum tolerated dose (MTD) of
approximately 1 hour duration were planned for each patient on
study days 1 through 10 of each 31 day cycle. Treatment could be
held up to 3 days for the occurrence of drug-related toxicity of
grade III or greater.

Protocol design. The protocol was a multi-center dose-escalation
study designed to establish the MTD. It was performed under a
US Investigational New Drug Application (IND), and was
approved by the Investigational Review Board of each institution.
Written informed consent was obtained from each patient before
entry into the study.

Eligibility criteria for patient entry into the study included
histologic evidence for CTCL, or Sezary syndrome, and expression
of the CD5 antigen by the tumor cells (Fig 1). Patients had to be
over 18 years of age, have a life expectancy of 3 months, have a
performance status of greater than 70% (Karnofsky), and have
measurable disease. Patients were not eligible if they had not been
offered standard therapy, if they had known sensitivity to mouse
proteins, if they had previous MoAb therapy, or if they had serious
organ dysfunction.

Patients were treated in groups of three to establish the MTD
with dose expansion at MTD. Before study entry, patients were
registered and assigned a dose level. Each treatment cycle lasted
31 days, with the first cycle consisting of immunotoxin administered
on days 1 through 10. Patients could be retreated 20 days after the

![Fig 1. Skin biopsy of cutaneous tumor of patient 6. (A) Hematoxylin and eosin stain showing malignant cell infiltrates. (B) Indirect avidin-biotin immunoperoxidase staining of the same tumor section. Sections were incubated first with H65-MoAb, then with biotinylated goat antimouse sera.](image-url)
last infusion for two additional cycles. A complete tumor assessment, physical examination, urinalysis, serum chemistry, complete blood count (CBC) with platelet count, Sezary count (if applicable), immunophenotype, quantitative Ig, and coagulation profile were obtained before infusion. Patients were evaluated during therapy with a daily physical examination. Serum chemistries, CBC, and urinalysis were obtained on study days 1, 4, 7, 11, and 22. Tumor measurements were obtained on day 21 of each cycle, or every 6 weeks. Sezary count was measured in appropriate patients on days 10 and 21 of each cycle, and day 42 of the final cycle. Quantitative Igs were performed on day 31 of each cycle. At each dose level, patients were evaluated for safety and response. Toxicities were graded according to standard criteria (World Health Organization [WHO]).

While the major thrust of this study was to establish the MTD, attempts were also made to evaluate efficacy. The following response criteria were used.

Complete remission was defined as the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks, with no symptoms attributable to tumor.

Partial remission was defined as a 50% or greater reduction in the sum of all measurable lesions, with no simultaneous increase in the size of any lesion or the appearance of new lesions for a minimum of 4 weeks. Sezary cells must decrease to less than 20% of baseline, and remain at that level for 4 weeks.

Stable disease was defined as no change or less than 50% reduction in the tumor size. In the case of Sezary syndrome, stable disease was defined as no change in the number of Sezary cells from baseline.

Progressive disease was defined as an increase by 25% or greater in the size of any measured lesion or appearance of a new lesion. In the case of Sezary syndrome, an increase in the number of Sezary cells from baseline was considered to be progressive disease.

Pharmacokinetics. The level of immunoconjugate in serum was measured at multiple timepoints: before infusion, immediately following infusion, and at 5, 15, 30, 60, 120, 240, and 480 minutes after the end of the infusion on the first and last day of each treatment cycle. The level was measured by an enzyme immunoassay. Affinity-purified goat anti-RTA was adsorbed to polystyrene microliter plates (Dynatek Immunolon 2; Dynatek, Chantilly, VA) at 4°C overnight. These plates were then coated with bovine serum albumin (BSA). A standard H65-RTA preparation was prepared in normal human serum and diluted to provide 10 concentrations ranging from 0.61 to 312 ng/mL. Standards, assay controls, and patient samples were plated as triplicates at dilutions of 1:10 to 1:1,280 and incubated for 1 hour at 37°C. The probe, alkaline phosphatase-conjugated goat antihuman IgG, was plated, incubated at 37°C for 1 hour, and developed for an additional 1 hour at room temperature after adding p-nitrophenylphosphate; absorbance was read at 405 nm, and levels of immunoconjugate were expressed as the dilution at which a line drawn through the most linear portion of the plot of optical density (OD) versus log dilution intersected the horizontal 0.1 OD line. To obtain response ratio, endpoint titer was divided by pretreatment titer. Assay reproducibility produced a coefficient of variation of 10.9%.

Blocking antibodies. Presence or absence of antibodies presumed to be directed against epitopes near the binding site of the MoAb portion of the immunoconjugate, and therefore capable of preventing binding, was quantitated by measuring binding to MOLT-4 target cells of fluorescein-labeled H65 MoAb in the presence of various dilutions of patient's sera.\(^{7,9}\) Briefly, MOLT-4 cells were washed and suspended at a concentration of 10^6 cells/mL in 1 mL of phosphate-buffered saline, to which 0.5, 10, 20, or 40 μL of patient serum was added. To this mixture 1 μg of H65-fluorescein isothiocyanate (FITC) was added, and the mixture was incubated for 1 hour at 4°C. Fluorescence intensity of the cells was assessed by flow cytometry. Data are expressed as the inverse titer of patient serum producing 50% inhibition of the fluorescence intensity of preparations containing no serum. Titers of 25 or greater were considered indicative of the presence of blocking antibodies.

Lymphocyte phenotype. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque density gradient centrifugation. Total T and B lymphocytes were identified by direct immunofluorescence using an anti-CD3 (Leu-4) and an anti-CD19 antibody (Leu-12; Becton Dickinson Co, Mountain View, CA). CD5 bearing T and B lymphocytes were enumerated by double-immunofluorescence flow cytometry using Leu-1 (anti-CD5) in conjunction with Leu 4 or B1, and converted to absolute numbers. Samples were analyzed within 24 hours of collection, and samples of PBMC containing less than 95% lymphocytes were excluded from analysis. This measurement was run on days 1 (pretreatment), 2, 3, 8, 11, and 21 of each cycle, and 3 months after final treatment.

RESULTS

Patient characteristics. The pretreatment characteristics of the 14 patients treated in this phase I study are listed in Table 1. The eight men and six women had a median age of 63 years (range, 24 to 69 years). The patients had an excellent median performance status of 90% despite an advanced stage of disease (median stage III). All patients had histologically documented cutaneous involvement, with half of the patients demonstrating extracutaneous disease.

The median time from diagnosis was 3 years and all patients had received multiple other therapies without success.

Dose and toxicity. Fourteen patients were treated with a median cumulative dose of 280 mg (range, 135 to 845 mg) of immunoconjugate. Twenty-one treatment cycles were attempted; five patients received less than one full cycle, eight received one to two cycles, and one received three cycles. Three patients were treated at 0.2 mg/kg/d, five at 0.33 mg/kg/d, and three at 0.5 mg/kg/d; none of the last dose group completed the 10 infusions. Three additional patients were then treated at 0.33 mg/kg/d, for a total of eight at this dose.

The H65-RTA infusions were usually well tolerated when administered at the MTD (Fig 2). The MTD was defined in this study as 0.33 mg/kg/d for 10 days. At the dose level above the MTD, two patients developed reversible
grade III dyspnea. This shortness of breath was first noted in a mild form at the 0.33 mg/kg dose level, began soon after infusion, reversed within 45 minutes to 36 hours after stopping the drug, and was not associated with signs of congestive heart failure or pulmonary edema. This event was considered mild and transient, allowing for continued dose escalation. However, at the higher dose level, two of the three patients experienced severe dyspnea, one associated with mild pulmonary edema observed on chest radiograph, lasting up to 10 days after discontinuation of the immunoconjugate. This toxicity was felt to be dose-related and established the MTD. One additional patient treated at the 0.5 mg/kg level developed a rash and mild coagulopathy, which later was found to be associated with cryofibrinogenemia, unrelated to drug administration.

Edema formation associated with weight gain of more than 2 kg was seen in 18 of 21 cycles of the drug. The degree of weight gain was classed as a grade III toxicity in one patient treated at 0.5 mg/kg/d (patient 11), and limited completion of the second cycle in a second patient treated at this dose (patient 14). A decrease in serum albumin from 10% to 45% of baseline was observed in all 21 treatment cycles, but was self-limited and reversed after completion of the H65-RTA. The serum albumin could be maintained above 3.0 g/dL in all patients who received supplemental albumin infusions. Other side effects, such as chills (43%), fever >38.0°C (38%), nausea (24%), fatigue (24%), and myalgia-arthritisgia (19%), were similar to those seen with other immunoconjugates. These toxicities were all grade II or less, and none were dose limiting.

Six of the 14 patients received more than one cycle of immunoconjugate therapy (Table 2). With the first dose of the second cycle, one patient developed hives and periorbital edema, not associated with shortness of breath and controlled with diphenhydramine. This patient safely received three additional doses after premedication with acetaminophen and diphenhydramine, but requested to be removed from the study so that she might receive a bone marrow transplant. The other patients were safely retreated with side effects similar to those experienced in their first treatment cycle. All of these patients developed detectable anti-immunoconjugate antibody responses.

There was one death on study, occurring in a patient who developed an infection of a central venous catheter. Sepsis due to Acinetobacter was documented antemortem and treated with antibiotics. The patient appeared to respond initially to therapy, but then suddenly developed septic shock. At postmortem examination, the patient was found to have blood cultures positive for Acinetobacter and to have

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**Table 1. Patient Demographics and Dose**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Daily Dose (mg/kg)</th>
<th>Stage</th>
<th>Disease Sites</th>
<th>Diagnosis Time (y)</th>
<th>Last Rx (mol)</th>
<th>Previous Therapy</th>
<th>Performance Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (181)</td>
<td>45/M</td>
<td>0.2</td>
<td>IVa</td>
<td>E, P, N</td>
<td>5.00</td>
<td>11</td>
<td>C, T, B, R, P, I</td>
<td>90</td>
</tr>
<tr>
<td>2 (162)</td>
<td>24/F</td>
<td>0.2</td>
<td>IVa</td>
<td>E, N</td>
<td>3.00</td>
<td>5</td>
<td>C, T, P, I</td>
<td>90</td>
</tr>
<tr>
<td>3 (101)</td>
<td>67/F</td>
<td>0.2</td>
<td>IB</td>
<td>P</td>
<td>8.50</td>
<td>6</td>
<td>C, B, P, Ph, I</td>
<td>100</td>
</tr>
<tr>
<td>4 (163)</td>
<td>62/M</td>
<td>0.33</td>
<td>IVb</td>
<td>P, N, T, BM, S</td>
<td>2.00</td>
<td>1</td>
<td>C, T, P, Ph</td>
<td>80</td>
</tr>
<tr>
<td>5 (131)</td>
<td>49/M</td>
<td>0.33</td>
<td>IIb</td>
<td>P</td>
<td>1.25</td>
<td>2</td>
<td>P, I</td>
<td>90</td>
</tr>
<tr>
<td>6 (102)</td>
<td>63/M</td>
<td>0.33</td>
<td>III</td>
<td>E, N</td>
<td>0.75</td>
<td>5</td>
<td>T, I</td>
<td>80</td>
</tr>
<tr>
<td>7 (103)</td>
<td>65/M</td>
<td>0.33</td>
<td>III</td>
<td>P</td>
<td>6.50</td>
<td>4</td>
<td>C, T, B, P, I</td>
<td>100</td>
</tr>
<tr>
<td>8 (164)</td>
<td>36/F</td>
<td>0.33</td>
<td>IVb</td>
<td>P, N, T, BM</td>
<td>7.75</td>
<td>1</td>
<td>C, T, B, I</td>
<td>100</td>
</tr>
<tr>
<td>9 (165)</td>
<td>67/F</td>
<td>0.5</td>
<td>IVb</td>
<td>P, N</td>
<td>2.00</td>
<td>5</td>
<td>C</td>
<td>70</td>
</tr>
<tr>
<td>10 (191)</td>
<td>64/M</td>
<td>0.5</td>
<td>III</td>
<td>P</td>
<td>3.00</td>
<td>1</td>
<td>T, R, P, I</td>
<td>90</td>
</tr>
<tr>
<td>11 (192)</td>
<td>64/M</td>
<td>0.5</td>
<td>III</td>
<td>P</td>
<td>1.25</td>
<td>12</td>
<td>R</td>
<td>90</td>
</tr>
<tr>
<td>12 (132)</td>
<td>69/F</td>
<td>0.33</td>
<td>III</td>
<td>C, S</td>
<td>2.50</td>
<td>1</td>
<td>P, I, Ph</td>
<td>90</td>
</tr>
<tr>
<td>13 (133)</td>
<td>63/F</td>
<td>0.33</td>
<td>III</td>
<td>C</td>
<td>1.75</td>
<td>1</td>
<td>P, I, Ph</td>
<td>90</td>
</tr>
<tr>
<td>14 (166)</td>
<td>61/M</td>
<td>0.33</td>
<td>IVb</td>
<td>Lung, C, N, BM</td>
<td>1.75</td>
<td>5</td>
<td>C, B</td>
<td>70</td>
</tr>
</tbody>
</table>

Demographics are given in order of treatment.

Abbreviations: E, erythroderma; P, plaque; N, node; BM, bone marrow; S, Sezary syndrome; B, electron beam; R, radiotherapy; P, psoralen-ultraviolet A (PUVA); Ph, photopheresis; C, systemic chemotherapy; T, topical chemotherapy; I, interferon.

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**Fig 2. Clinical events associated with H65-RTA administration.**

Fourteen patients received 21 partial or complete cycles of drug with 10 days per cycle. All patients who received a second cycle had completed the first, although in two cases less than 10 doses were administered in the second cycle. All events noted occurred during or within 7 days of the infusion cycle, and all reversed within 3 weeks except for peripheral edema in patient 191, which required 35 days to reverse. Most side effects were grade II by WHO criteria. The MTD was defined by the two patients with dyspnea, treated at the 0.5 mg/mL dose level.
were found in seven. Blocking antibodies first appeared in patients not retreated, the titers began to decline within 10 days after treatment, but retreatment was usually associated with increasing titers. Sera from 11 patients were evaluated for blocking antibodies, and significant titers were found in seven. Blocking antibodies first appeared about day 15 in one patient, about day 30 in two, day 40 in one, and day 60 in three.

Pharmacokinetics. Pharmacokinetic data for the first and or last infusions of the first cycle of H65-RTA are available in 13 patients, for the second cycle in six patients, and for the third cycle in one patient. Values for the first and last infusion of the first cycle and first infusion of the second cycle are shown in Table 4; samples were only received for the second infusion of the second cycle in two patients (these values are not shown). Peak serum levels, ranging from 1.13 to 5.65 μg/mL, were usually achieved immediately at the end of the infusion on the first day of treatment. By day 10, most patients had marked reductions in drug levels, usually associated with the development of anti-immunoconjugate antibodies (Fig 3, Table 3), in which antibody titers greater than 1:2,000 were correlated with peak drug levels of less than 0.5 μg/mL. The exception to this observation was patient 4, whose initial peak serum level was 0.38 μg/mL, probably due to the absorption of immunoconjugate by large numbers of circulating Sezary cells. Peak drug levels in this patient actually increased with retreatment, in concert with a decline in circulating tumor cells. During the first cycle the drug had a median THL (T1/2P) of 2.0 hours (range, 1.2 to 2.9). In patients in whom it could be assessed, the THL was not significantly altered from the first to the tenth day of infusion.

Lymphocyte immunophenotype. Of the 12 patients evaluated for CD3+/CD5+ lymphocytes in the first treatment cycle, three had Sezary cells in the circulation and were evaluated separately. Two patients treated above the MTD received only four doses of H65-RTA (data not shown). Patient 9 (165) had a transient decrease of CD3+/CD5' lymphocytes below 25% of baseline with rapid recovery to above baseline on treatment day 8. Patient 10 (191) also received four doses of H65-RTA, but sustained a prolonged suppression of CD3+/CD5' cells below 30% of baseline with recovery documented on day 32.

Data from the remaining seven patients are depicted in Fig 4. All patients except one demonstrated significant reductions in CD3+/CD5+ lymphocytes with a nadir ranging from the day 1 to 7 of treatment. Patient 1 (161) displayed a paradoxical increase in CD3+/CD5' cells during therapy. Despite the impressive increase in these cells to greater than three times baseline, the CD3'/CD7' populations declined to less than 10% of their pretreatment values.

Clinical response. Of the 14 patients treated on this study, there were four partial responses lasting from 3 to 8 months (Table 5). These responses were not clearly related to dose of H65-RTA, but two of the responses occurred at the dose level above the MTD. Patients 1 and 4 had more than one cycle of immunoconjugate therapy with continued improvement in their disease. Patient 1 had only a minimal response after the first cycle of therapy, which improved to a partial response with a second treatment. This patient’s indicator lesions completely resolved despite the presence of an anti-immunoconjugate titer of 1:60,000. Biopsy of the skin in the area of the tumor showed residual malignant lymphocytes that expressed the CD5 antigen by immunostaining. A similar pattern of response was seen in patient

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**Table 2. Relationship of Antibody Titer and Side Effects in Patients With H65-RTA**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Site of Disease</th>
<th>Dose (kg/mg/d)</th>
<th>Cycles</th>
<th>Antibody Titer at</th>
<th>Allergic Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (161)</td>
<td>45/M</td>
<td>Cutaneous</td>
<td>0.2</td>
<td>2</td>
<td>60,000</td>
<td>None</td>
</tr>
<tr>
<td>3 (101)</td>
<td>67/F</td>
<td>Cutaneous</td>
<td>0.2</td>
<td>2</td>
<td>28,000</td>
<td>None</td>
</tr>
<tr>
<td>4 (163)</td>
<td>62/M</td>
<td>Sezary cells</td>
<td>0.33</td>
<td>3</td>
<td>600/1,100</td>
<td>None</td>
</tr>
<tr>
<td>8 (164)</td>
<td>36/F</td>
<td>Cutaneous</td>
<td>0.33</td>
<td>1.4</td>
<td>33,000</td>
<td>Periorbital edema, hives, during cycle 2</td>
</tr>
<tr>
<td>12 (132)</td>
<td>69/F</td>
<td>Sezary cells</td>
<td>0.33</td>
<td>2</td>
<td>1,900,000</td>
<td>None</td>
</tr>
<tr>
<td>14 (166)</td>
<td>59/F</td>
<td>Cutaneous</td>
<td>0.33</td>
<td>1.9</td>
<td>130,000</td>
<td>None</td>
</tr>
</tbody>
</table>

**Table 3. Anti-Immunoconjugate (IgG) and Anti-Idiotypic Antibody Titers**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Dose</th>
<th>Peak Titer*</th>
<th>Blocking Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μg)</td>
<td>(d)</td>
<td>Titer</td>
</tr>
<tr>
<td>1 (161)</td>
<td>0.2</td>
<td>580,000</td>
<td>None</td>
</tr>
<tr>
<td>2 (162)</td>
<td>0.2</td>
<td>320 (21)</td>
<td>NA</td>
</tr>
<tr>
<td>3 (101)</td>
<td>0.2</td>
<td>800,000</td>
<td>83</td>
</tr>
<tr>
<td>4 (163)</td>
<td>0.33</td>
<td>10,000,000</td>
<td>89</td>
</tr>
<tr>
<td>5 (131)</td>
<td>0.33</td>
<td>135,000</td>
<td>40</td>
</tr>
<tr>
<td>6 (102)</td>
<td>0.33</td>
<td>91,000</td>
<td>110</td>
</tr>
<tr>
<td>7 (103)</td>
<td>0.33</td>
<td>120,000</td>
<td>110</td>
</tr>
<tr>
<td>8 (154)</td>
<td>0.33</td>
<td>840,000</td>
<td>110</td>
</tr>
<tr>
<td>9 (165)</td>
<td>0.5</td>
<td>100 (1-174)</td>
<td>NT</td>
</tr>
<tr>
<td>12 (132)</td>
<td>0.33</td>
<td>1,900,000</td>
<td>35</td>
</tr>
<tr>
<td>13 (133)</td>
<td>0.33</td>
<td>2,800,000</td>
<td>57</td>
</tr>
<tr>
<td>14 (166)</td>
<td>0.33</td>
<td>820,000</td>
<td>57</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not applicable; NT, not tested.

*Days of follow-up ranged from 20 to 174.
cycles of therapy (Fig 5). This patient also produced a
initiating H65-RTA therapy, but usually recurred before
mor activity that did not meet the criteria for a response
determination (number, quantity, etc).

This patient experienced marked reduction in the percent-
skin disease, and patient 9 also had greater than 75%
symptoms. All patients had improvement in erythroderma
occurred in a patient with Sezary syndrome (patient 13).

and pruritus that typically began within a few days of
PCNONLIN; 20-fold higher on the last dose of the first cycle
\( \text{titer of most proximate sample.} \)

\[ \frac{14}{10} \]

While the cutaneous T-cell lymphomas represent a
heterogeneous group of diseases, they share the common
features of a T-lymphocyte phenotype of the tumor cell, a
propensity for skin infiltration, and early systemic involve-
ment. Traditional therapies have addressed local control of
cutaneous disease, but these ultimately fail because of
disseminated disease. Systemic chemotherapies have been
reserved for more advanced disease, but can be compli-
cated to administer in this setting because of significant
toxicities, especially sepsis associated with skin ulceration.

Recently, combined modality therapy using systemic elec-
tron beam therapy and systemic chemotherapy has been
shown to be feasible in patients with no prior treatmenL3
However, when compared with topical chemotherapy, ther-
apy-related toxicity was considerably higher without a
benefit in disease-free survival. Ideally, the new therapies
developed for treatment of CTCL would address the early,
subclinical dissemination of the disease, would display
minimal systemic toxicity (especially myelosuppression),
and would complement present treatment modalities
through a different mechanism of action. H65-RTA is an
appropriate candidate for such a therapeutic agent.

This phase I trial defines the side effects and maximal
tolerated dose of H65-RTA when administered as a 1-hour
IV infusion in patients with CTCL. The recommended dose
for phase II trials established in this study was 0.33 mg/kg/d
for 10 days. At the dose level above this, two patients
experienced reversible grade III dyspnea with one patient
developing pulmonary edema. Side effects below the MTD
were characteristic of those described with this and other
immunoconjugates.12-23 The development of hypoalbumin-
emia, weight gain, and edema has been observed with RTA
immunoconjugates in the therapy of melanoma and colon
cancer, but has not been observed with antibody alone.21,23

Table 4. Relationship Between Antibody Titers and Pharmacokinetics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Dose (mg/kg)</th>
<th>Antibody* Titer</th>
<th>PDL/THL</th>
<th>Antibody* Titer</th>
<th>PDL/THL</th>
<th>Antibody* Titer</th>
<th>PDL/THL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (161)</td>
<td>0.2</td>
<td>100</td>
<td>5.65/2.0</td>
<td>200</td>
<td>1.29/2.5</td>
<td>60,000</td>
<td>0.08/ND</td>
</tr>
<tr>
<td>2 (162)</td>
<td>0.2</td>
<td>100</td>
<td>1.47/1.6</td>
<td>100</td>
<td>1.13/1.2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3 (101)</td>
<td>0.2</td>
<td>100</td>
<td>1.14/2.6</td>
<td>2,000</td>
<td>0.5/TL</td>
<td>28,000</td>
<td>0.07/ND</td>
</tr>
<tr>
<td>4 (163)</td>
<td>0.33</td>
<td>100</td>
<td>0.38/TL</td>
<td>1,200</td>
<td>NS</td>
<td>600</td>
<td>2.50/1.0</td>
</tr>
<tr>
<td>5 (131)</td>
<td>0.33</td>
<td>100</td>
<td>3.44/2.9</td>
<td>135,000</td>
<td>0.01/TL</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6 (102)</td>
<td>0.33</td>
<td>100</td>
<td>2.23/1.2</td>
<td>76,000</td>
<td>0.12/TL</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7 (103)</td>
<td>0.33</td>
<td>1,100</td>
<td>3.9/2.6</td>
<td>40,000</td>
<td>NS</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8 (164)</td>
<td>0.33</td>
<td>100</td>
<td>2.87/1.8</td>
<td>1,000</td>
<td>1.68/IS</td>
<td>33,000</td>
<td>0.16/TL</td>
</tr>
<tr>
<td>12 (132)</td>
<td>0.33</td>
<td>580</td>
<td>3.86/2.6</td>
<td>580</td>
<td>4.0/IS</td>
<td>&gt; 39,000</td>
<td>0.20/TL</td>
</tr>
<tr>
<td>13 (133)</td>
<td>0.33</td>
<td>180</td>
<td>2.82/1.3</td>
<td>180</td>
<td>2.66/1.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>14 (166)</td>
<td>0.33</td>
<td>100</td>
<td>NS</td>
<td>56,000</td>
<td>NS</td>
<td>130,000</td>
<td>0.02/TL</td>
</tr>
</tbody>
</table>

Abbreviations: PDL, peak drug level, usually at end of infusion (when sample available); THL, terminal half-life (h), two-compartment analysis using PCNONLIN; –, no second cycle; TL, concentration too low for half-life determination; NS, no sample received; IS, sample not sufficient for half-life determination (number, quantity, etc).

*Titer of most proximate sample.

Fig 3. Relationship of pharmacokinetics of H65-RTA and antibody titer. Patient 3[101], receiving two cycles of 0.2 mg/kg/d of H65-RTA, developed progressively increasing titers of anti-immunoconjugate antibody. Antibody titers were baseline on the first infusion of the first cycle (■), 20-fold higher on the last dose of the first cycle (■), and over 100 times higher on the first day of the second cycle (■).
The toxicity profile displayed by H65-RTA at the MTD allows the drug to be administered on an outpatient basis, and does not overlap with the toxicities of other agents active in this disease. Previous studies of immunoconjugates in cancer therapy have been restricted to one course of treatment. For a targeted therapy such as H65-RTA to be effective in the treatment of CTCL, it is likely that multiple cycles of treatment will be needed. An important observation from this study is that six of the patients could be retreated without additional toxicities. Only one patient displayed allergic manifestations of hives and periorbital edema. These symptoms did not prevent continued treatment after premedication with diphenhydramine and acetaminophen.

Table 5. Response of Patients Treated With H65-RTA

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Dose (mg/kg/d)</th>
<th>No. of Infusions</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
<th>Response</th>
<th>Duration (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (161)</td>
<td>0.2</td>
<td>20</td>
<td>Single skin lesion</td>
<td>Complete clearing</td>
<td>Partial</td>
<td>3</td>
</tr>
<tr>
<td>2 (162)</td>
<td>0.2</td>
<td>10</td>
<td>11 cm² skin lesion</td>
<td>Decrease to 8.9 cm²</td>
<td>Stable</td>
<td>—</td>
</tr>
<tr>
<td>3 (101)</td>
<td>0.2</td>
<td>20</td>
<td>6.7 cm² skin lesion</td>
<td>7.8 cm² skin lesion</td>
<td>Stable</td>
<td>—</td>
</tr>
<tr>
<td>4 (163)</td>
<td>0.33</td>
<td>30</td>
<td>Sezary cells</td>
<td>Complete clearing</td>
<td>Partial</td>
<td>4</td>
</tr>
<tr>
<td>5 (131)</td>
<td>0.33</td>
<td>10</td>
<td>Total lesion size 303 cm²</td>
<td>Total lesion size 279 cm²</td>
<td>Progressive</td>
<td>—</td>
</tr>
<tr>
<td>6 (102)</td>
<td>0.33</td>
<td>9</td>
<td>Total body erythroderma</td>
<td>Appearance of new tumors</td>
<td>Stable</td>
<td>—</td>
</tr>
<tr>
<td>7 (103)</td>
<td>0.33</td>
<td>8</td>
<td>Skin lesion 1.75 cm²</td>
<td>Skin lesion 2.8 cm²</td>
<td>Progressive</td>
<td>—</td>
</tr>
<tr>
<td>8 (164)</td>
<td>0.33</td>
<td>14</td>
<td>Skin lesion; cycle 1, 20 cm²; cycle 2, 19.55 cm²</td>
<td>Skin lesion: cycle 1, 19.55 cm²; cycle 2, 36.62 cm²</td>
<td>Progressive</td>
<td>—</td>
</tr>
<tr>
<td>9 (165)</td>
<td>0.5</td>
<td>4</td>
<td>Two skin lesions</td>
<td>Completely resolved</td>
<td>Partial</td>
<td>8</td>
</tr>
<tr>
<td>10 (191)</td>
<td>0.5</td>
<td>4</td>
<td>Multiple skin lesions 150.7 cm²</td>
<td>Decreased in size by 75%</td>
<td>Partial</td>
<td>3</td>
</tr>
<tr>
<td>11 (192)</td>
<td>0.5</td>
<td>4</td>
<td>No follow-up</td>
<td>Not available</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>12 (132)</td>
<td>0.5</td>
<td>20</td>
<td>Sezary cells: 70% cycle 1; 75% cycle 2</td>
<td>Sezary cells: 70% cycle 1; 75% cycle 2</td>
<td>Stable</td>
<td>—</td>
</tr>
<tr>
<td>13 (133)</td>
<td>0.33</td>
<td>10</td>
<td>50% Sezary cells*</td>
<td>40% Sezary cells</td>
<td>Stable</td>
<td>—</td>
</tr>
<tr>
<td>14 (166)</td>
<td>0.33</td>
<td>19</td>
<td>11 cm² skin lesion</td>
<td>4 cm² skin lesion, new skin lesion</td>
<td>Progressive</td>
<td>—</td>
</tr>
</tbody>
</table>

*Transient decrease to 14% day 14.
One death occurred in this study, a patient with advanced CTCL and marked skin ulceration and superinfection. This patient developed a catheter-related infection, not associ-
ated with the study drug, and subsequently died of septic shock. Unfortunately, sepsis remains a major complication of this malignancy and an important cause of death in
advanced disease. 25,26

Pharmacokinetic analysis is available for the first and last infusions of the first cycle in 10 patients, and for the second cycle in three patients. In all patients except one, the peak serum drug concentrations occurred immediately after completion of the infusion. One patient with Sezary syn-
drome developed progressively higher peak drug levels as
the leukemic cells were cleared from the blood. Presum-
ably, the H65-RTA was absorbed on to the malignant cells immediately and was therefore not detectable. The immu-
nocojugate displayed a biphasic clearance, similar to that
described in patients with graft-versus-host disease (GVHD),16 with a terminal phase of 2.0 hours. Schedules developed with consideration of the relatively short half-life
may prove to be more active than a single daily infusion. The development of anti-immunoconjugate antibodies did significantly affect the peak serum concentrations as early as the 10th dose, even though the THL was not affected.

In contrast to the patients treated with H65-RTA for steroid refractory acute GVHD,15 patients with CTCL developed a significant anti-immunoconjugate response. Of
the patients examined for the presence of anti--H65-RTA antibodies and for blocking antibodies, 10 of 12 had significant antibody titers against the immunoconjugate and seven had blocking antibodies. Neither the presence of anti-immunoconjugate antibody nor blocking antibody was associated with enhanced toxicity. Two patients with signif-
icant titers of anti-immunoconjugate antibody, but without blocking antibody, were also found to have continued antitumor response with repeated administration of the
drug. In contrast, none of the seven patients who developed blocking antibody responded to treatment. This result suggests that the formation of anti-immunoconjugate anti-
bodies should not necessarily be viewed as an absolute contraindication to immunoconjugate therapy. However, it
may be necessary to treat such patients with higher doses or prolonged courses of immunoconjugate to overcome the effect of such antibodies. The preservation of antitumor activity in the presence of antidrug antibody had been observed in preclinical models with interferon and cisplati-
num. 27,28

Twelve patients were evaluated for PB lymphocyte (PBL)
phenotype during the first course of therapy. Of the seven evaluable patients, six had a marked decline in CD3+/CD5+ lymphocytes during treatment, with rapid restoration of lymphocyte counts after completion of therapy. Restora-
tion of pretreatment lymphocyte values in the face of
continuing resolution of tumor suggests the sensitivity of
tumor cells to H65-RTA may be different than that of normal lymphocytes. In fact, patient 1 demonstrated no
 clearing of PBL, but achieved a partial antitumor response.
Both patients with Sezary syndrome exhibited significant reductions in peripheral malignant cell numbers. While the
potential for suppression of the immune system theoreti-
cally exists with an immunoconjugate that recognizes nor-
mal T cells, no clinical evidence of drug-induced immuno-
suppression was observed in these patients. Only one
infection occurred on study, which was a bacterial infection thought to be related to catheter sepsis.

In addition to the primary dose-finding objective of this
phase I trial, there was an opportunity to observe antitumor activity. As has been described for unconjugated antibod-
ies, virtually all patients had at least transient improvement
in erythroderma and pruritus associated with treatment. 9,11
In addition, four of the patients achieved a reduction of measurable tumor burden by 50% or greater lasting from 3
to 8 months. It should be pointed out that partial responses
have also been achieved with unconjugated anti-CD5 anti-
bodies, but these are infrequent and usually transient. 29
From this study it is not possible to conclude whether the
H65-RTA is associated with an improved response rate over unconjugated antibodies. What is evident is that the
tumors of patients who have extensive therapy remain
sensitive to the cytotoxic effects of H65-RTA.

An important consideration in immunoconjugate therapy
is the impact of antibody response on antitumor activity.
Two of the patients who achieved a partial response did so
only after more than one treatment cycle. In both cases, the
patients had significant levels of anti-immunoconjugate antibody, although neither had blocking antibodies. Bec-
cause the CD5 antigen is not shed into the circulation,
nonspecific binding to circulating antigen was not an
impediment to tumor therapy.

In summary, H65-RTA is an active drug in the treatment
of CTCL. Phase II trials will be required to accurately
identify the response rate in this disease. From the present study, we would recommend 0.33 mg/kg/d as the starting dose for such trials. At this dose, the immunonoconjugate is safe and associated only with mild reversible side effects, allowing for outpatient therapy. The development of host anti-immunoconjugate responses does not necessarily mandate the termination of therapy, but is associated with lower peak serum drug levels. Because of the relatively short half-life, additional trials investigating different schedules are warranted. Finally, the non-overlapping toxicities with currently used chemotherapeutic agents make H65-RTA an attractive candidate for combination therapy of systemic disease.

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Phase I trial of H65-RTA immunoconjugate in patients with cutaneous T- cell lymphoma

CF LeMaistre, S Rosen, A Frankel, S Kornfeld, E Saria, C Meneghetti, J Drajesk, D Fishwild, P Scannon and V Byers