Resimmune, an anti-CD3ε recombinant immunotoxin, induces durable remissions in patients with cutaneous T-cell lymphoma

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ABSTRACT

Resimmune is a second-generation recombinant immunotoxin composed of the catalytic and translocation domains of diphtheria toxin fused to two single chain antibody fragments reactive with the extracellular domain of CD3ε. We gave intravenous infusions of Resimmune 2.5 - 11.25 μg/kg over 15 minutes to 30 patients (25 with cutaneous T-cell lymphoma, 3 with peripheral T-cell lymphoma, 1 with T-cell large granular lymphocytic leukemia and 1 with T-cell prolymphocytic leukemia) in an inter-patient dose escalation trial. The most common adverse events were fever, chills, hypotension, edema, hypoalbuminemia, hypophosphatemia, and transaminasemia. Among the 25 patients with cutaneous T-cell lymphoma, there were nine responses for a response rate of 36% (95% CI, 18%-57%) including four complete remissions (16%, 95% CI, 5%-36%). The durations of the complete remissions were 72+, 72+, 60+ and 38+ months. There were five partial remissions lasting 3, 3, 3+, 6+ and 14 months. Of 17 patients with a modified skin weighted assessment tool score <50, 17 patients with stage IB/IIB, and 11 patients with both a score <50 and stage IB/IIB, nine (53%), eight (47%), and eight (73%) had responses, respectively. Further studies of Resimmune in patients with low tumor burden, stage IB-IIB cutaneous T-cell lymphoma are warranted. This trial is registered at clinicaltrials.gov as #NCT00611208.

Introduction

Cutaneous T-cell lymphoma (CTCL), a malignancy of skin-tropic T cells, has an incidence of 2,400 cases per year in the USA.[1,2] Numerous topical and systemic therapies have been approved, including topical nitrogen mustard, oral bexarotene, romidepsin, and vorinostat, alemtuzumab, extracorporeal photopheresis, and allogeneic stem cell transplantation.[3-5] Most of the treatments are chronic or require multiple courses and physician visits. Side effects are considerable and range from local tissue injury to constitutional symptoms, organ injuries, immunosuppression, and graft-*versus*-host disease. While allogeneic stem cell transplantation may provide long-term remissions, most therapies yield responses lasting months. Overall, CTCL has a long clinical course with relentless progression over months to years for many patients with an estimated median survival of 3 to 5 years for stage IB-IIB patients.[4] We sought to identify a novel agent that could be given over a shorter treatment period than other anti-CTCL modalities, yield fewer prolonged side effects, and produce durable clinical benefit.

One such class of therapeutics is immunotoxins composed of lymphoma-selective ligands covalently linked to protein synthesis inactivating peptide toxins.[6] The ligand (or antibody) directs the molecule to the surface of the lymphoma cell. After ligand binding and internalization, the toxin escapes to the cytosol and catalytically inhibits protein synthesis leading to cell death. A series of immunotoxins have been clinically tested in T-cell malignancies including diphtheria toxin fused to human interleukin-2 (denileukin diftitox) and Pseudomonas exotoxin fused to an anti-CD25 antibody Fv (LMB-2). Several of these agents produced partial remissions in about one third of treated patients.

To improve the clinical benefit and broaden activity, we synthesized a second-generation immunotoxin, Resimmune or A-dmDT390-bisFv(UCHT1), consisting of the catalytic and translocation domains of diphtheria toxin (DT_{390}) fused to two single chain antibody fragments reactive with an acidic loop on the extracellular domain of CD3ε.[7] CD3ε is a component of the T-cell receptor.[8] The CD3 subunits are expressed on the vast majority of mature T-cell neoplastic cells.[9] Further, antibody cross-linking of CD3ε triggers efficient internalization of the complex yielding highly potent immuntoxins.[10]

Clinical material was prepared by expressing Resimmune in *Pichia pastoris* and purifying recombinant protein by anion exchange and hydrophobic interaction chromatography.[11] The compound was selectively toxic in tissue culture and depleted several logs of antigen-positive cells in blood, lymph nodes and spleen of transgenic mice. Resimmune bound only splenic lymphocytes among 18 normal human tissues, and mice, rats and monkeys given total doses of >200 μg/kg over 4 days showed only transient transaminasemia without histopathological tissue injury or clinical signs or symptoms.[12] Based on these results, we were granted approval from the Food and Drug Administration to test this immunotoxin in patients with T-cell neoplasms (BB IND#100712). The starting dose (2.5 μg/kg x8) was one-tenth the maximum tolerated dose observed in monkeys.[12] This report describes the results of this study.

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Methods

The Resimmune study was a single-arm, multicenter inter-patient dose escalation phase 1 trial in patients with advanced CD3+ T-cell malignancies. The study was performed under the sponsorship of Angimmune, LLC, registered at clinical trials.gov as NCT00611208, and approved by Institutional Review Boards at the participating institutions. Thirty patients were treated with a single course of Resimmune at doses ranging from 2.5 to 11.25 μg/kg intravenously twice daily for 4 days.

Eligibility and diagnosis

Patients with CD3+ T-cell malignancies, diagnosed by morphological, histochemical, and cell surface criteria, in whom systemic therapy had failed were eligible for the study.

Treatment

Resimmune was given at dose of 2.5, 5, 7.5, or 11.25 μg/kg twice daily (4-6 hours apart) for 4 consecutive days through a free flowing intravenous set over 15 minutes. In the dose escalation portion of the study, cohorts of three patients were treated at each dose level unless dose-limiting toxicity was observed in one patient in which case the cohort was expanded to six patients. Once two patients at a dose level experienced dose-limiting toxicity, the next lower dose level was the maximum tolerated dose. In the expansion cohort, 13 additional CTCL patients were treated at the maximum tolerated dose of 7.5 μg/kg dose.

Toxicity and response evaluation

Toxicities were determined before treatment and daily for 4 days and then on days 10, 23, 37, and at follow-up visits by history, physical examinations, complete blood counts with differential, and serum chemistry. Electrocardiography was done before treatment and on days 1 and 4. Titers of Epstein-Barr virus (EBV) and cytomegalovirus (CMV) were determined by polymerase chain reaction analysis before treatment and on days 4, 10, 16, 23, and 37.

Table 1. Characteristics of the patients and their diseases.

Characteristics	Number of subjects (N=30)
Age, median (range), years	57 (20-84)
Gender (male/female)	12/18
Race	
Caucasians	19
Black	10
Hispanic	1
Disease	
Cutaneous T-cell lymphoma	25
Stage IB-IIB	17
Stage III-IV	8
Peripheral T-cell lymphoma	3
Prolymphocytic leukemia	1
Large granular lymphocytic leukemia	1
Prior therapy	
Lines, median (range)	3 (1-7)
Cytotoxic chemotherapy	17
Bexarotene	16
Interferon	7
Ionizing radiation	5
Romidepsin	4
Pralatrexate	3
Vorinostat	3
Alemtuzumab	2
Allogeneic hematopoietic cell transplant	2

Toxicities were graded using the revised National Cancer Institute Common Terminology Criteria for Adverse Events (*NCI CTCAE version 4.0; http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_201-06-14_QuickReference_5x7.pdf*).

CTCL response criteria were based on the modified skin-weighted assessment tool (mSWAT) score.[13] Complete response required an mSWAT score of 0, normal liver and spleen size, absence of pathological adenopathy as determined by clinical examination and computed tomography, and normal bone marrow biopsy and aspirate. A partial response required at least a 50% reduction in mSWAT with no new skin lesions and no pathological involvement of lymph nodes, bone marrow or visceral organs. Progressive disease was defined as a 25% increase in mSWAT or new non-skin disease. Stable disease was defined as the absence of complete remission, partial remission or progressive disease.

Pharmacology, immune response and flow cytometry

Resimmune concentrations in serum samples were measured by a bioassay using Jurkat cells.[14,15] Maximum concentration (C_{max}), serum half-life ($t_{½}$) and area under the curve (AUC) were determined. The immune response to Resimmune was measured by a sandwich enzyme immunoassay with a horseradish peroxidase-conjugated goat anti-human IgG. Human anti-DT antibody was purified from normal human serum using a Resimmune-conjugated sepharose affinity column and used as a standard for the anti-Resimmune antibody titer assay.[16] Flow cytometry was developed to monitor T-cell populations before and after immunotoxin therapy.[16]

Statistical analyses

Toxicities are dichotomized as none *versus* any or none and mild *versus* moderate and severe. The rates of toxicity, overall response, and complete response, as well as their 95% confidence intervals were estimated using an exact binomial method. The mean and standard deviation values of the pharmacokinetic parameters including C_{max} and $t_{1/2}$ are reported.

Results

Patients

Thirty patients were treated with 31 courses of Resimmune; one patient received a second course of treatment 6 months after disease recurrence. All 30 patients were evaluable for the safety analysis, whereas 26 patients were evaluable for objective response. Twenty-six patients received all eight doses in their first course, whereas one patient received a single dose, one patient received three doses, one patient received five doses, and one patient received six doses. The reasons for patients receiving fewer than eight doses during the treatment period were hypotension and hypoalbuminemia with or without hypoxia or congestive heart failure.

The patients' demographic data and prior treatment information are presented in Table 1 and *Online Supplementary Table S1*. There were 18 females and 12 males; the median age was 57 years (range, 20 to 84 years). The patients had received an average of three prior therapies, although four patients had received a single prior regimen and two patients had been treated with multiple modalities including allogeneic stem cell transplantation. Seventeen patients had CTCL stage IB-IIB; eight patients had CTCL stage III-IV; three patients had peripheral T-cell lymphoma; one patient had T-cell large granular lympho-

cytic leukemia and one patient had T-cell prolymphocytic leukemia. The study was subsequently modified to exclude patients with a prior history of heart disease and recent alemtuzumab therapy.

Toxicities

Adverse events attributed to drug treatment at the 2.5 – 11.5 μg/kg dose levels are listed in Table 2 and *Online Supplementary Table S2*. There were three grade 4/5 drug-related toxicities. Patient #10, at a dose of 5 μg/kg, developed severe congestive heart failure and vascular leak syndrome after five doses and died on day 11. He had a history of previous congestive heart failure and cardiomegaly. Patient #18, treated at 11.25 μg/kg, also developed severe congestive heart failure and vascular leak syndrome after six doses and died on day 9. She had a history of pulmonary hypertension and right ventricular dilatation. Patient #28 was treated with eight doses of 7.5 μg/kg and had EBV reactivation and EBV-induced liver and renal failure and died on day 29. He had received a course of alemtuzumab 4 months previously and had a $CD3^+CD4^+$ T-cell count of 211/μL prior to Resimmune. After these adverse events, the protocol was modified to exclude patients with a history of heart disease or recent alemtuzumab use. Additional grade 3 adverse events included six cases of EBV and/or CMV reactivation, two cases of hypophosphatemia, and two cases of transaminasemia. These toxicities were transient and treatable with rituximab, gancyclovir, phosphate replacement, or observation, respectively. Based on the occurrence of grade 3-5 toxicities in both patients at the 11.25 μg/kg dose level, the 7.5 μg/kg dose was chosen for the expansion cohort.

The most prominent side effect was vascular leak syndrome (VLS) associated with hypoalbuminemia, hypotension, fluid retention, edema, and, in some cases, heart failure. Ten patients had grade 2 VLS or hypoalbuminemia, and two patients with a history of heart disease had grade 4-5 VLS. Except in the two patients with heart failure, the VLS worsened over a week and then resolved over several more weeks. Supportive care with albumin infusions and diuretics reduced symptoms. Baseline albumin concentration was not predictive of VLS and steroids were not used for the management of the syndrome.

CMV and/or EBV reactivation, diagnosed on the basis of polymerase chain reaction assays, occurred in seven patients. In six patients, there were no associated symptoms, and the patients responded to gancyclovir given orally and/or rituximab administered intravenously. One patient with pretreatment lymphopenia after alemtuzumab therapy developed EBV viremia on day 24. He refused rituximab and died with multi-organ failure 5 days later.

Six patients experienced isolated elevation of hepatic transminases without hyperbilirubinemia. Four patients

Table 2. Treatment detaila and adverse events.

Cohort	Dose (μg/kg)	Doses received	Total dose in μg	N.	Drug-related adverse events (CTCAEv4.03 toxicity grade)	Dose-limiting toxicities
1	2.5	8	20	6	Grade 3 EBV/CMV infection (n=4) Grade 2 hypoalbuminemia (n=3) Grade 2 chills (n=3) Grade 2 AST, ALT elevations (n=2) Grade 2 fever (n=1) Grade 3 supraventricular tachycardia (n=1)	No
2	5	1-8	5-40	7	Grade 2 hypoalbuminemia (n=3) Grade 2-4 vascular leak syndrome (n=3) Grade 2 chills (n=2) Grade 2 hypotension (n=2) Grade 3 AST, ALT elevations (n=1) Grade 5 heart failure (n=1) Grade 3 uremia (n=1) Grade 3 hypophosphatemia (n=1) Grade 2 fever (n=1)	1
3	7.5	3-8	22.5-60	16	Grade 2 chills (n=5) Grade 2 fever (n=4) Grade 2-3 hypoalbuminemia (n=4) Grade 2-3 AST, ALT elevations (n=3) Grade 3 hypophosphatemia (n=2) Grade 2 hypomagnesemia (n=1) Grade 2 vascular leak syndrome (n=1) Grade 5 EBV infection (n=1) Grade 4 liver failure (n=1) Grade 4 uremia (n=1) Grade 4 metabolic acidosis (n=1)	1
4	11.25	6-8	67.5-90	2	Grade 5 heart failure (n=1) Grade 4 vascular leak syndrome (n=1) Grade 4 hypoxia (n=1) Grade 3 EBV infection (n=1) Grade 3 hypoalbuminemia (n=1) Grade 3 supraventricular tachycardia (n=1) Grade 4 hypotension (n=1) Grade 3 uremia (n=1)	1

had grade 2 elevations, and 2 patients had grade 3 elevations. The onset was generally on day 3 to 8, with complete resolution by days 15 to 21.

Four patients had transient electrolyte abnormalities during treatment including two patients with hypophosphatemia and one patient each with hypocalcemia and hypomagnesemia. Each patient responded quickly to electrolyte replacement.

Fourteen patients experienced transient infusion reactions several hours after infusion. All were mild to moderate in severity, possibly mitigated by the premedication regimen. Occasional patients required supplemental acetaminophen, meperidine, and/or H1 and H2-histamine antagonists. Symptoms included fever and/or chills. Three patients had transient hypotension, and one patient had hypoxemia. All these reactions resolved rapidly after administration of fluids or oxygen, respectively.

Pharmacological, immunological and flow cytometric studies

Serum samples were collected for pharmacokinetic studies on days 1 and 2 from 14 patients and for immune response measurements on day 1 from all 30 patients and on day 10 - 30 from 18 patients. Flow cytometry assays of circulating blood T cells were done on day 0 and day 4 or 5 for 20 patients. The results of the relevant pharmacological and immunological studies and circulating cell populations are shown in Table 3 and *Online Supplementary Tables S3-S5*. C_{max} values averaged 7.9 ng/mL (range, 0 to 41 ng/mL) after treatment on day 1. Drug levels were not detectable in samples from patients 8, 11, 13, 14, and 17. The clearance of Resimmune generally fitted a mono-exponential model. Drug clearance was highly variable with $t_{1/2}$ values averaging 39 min (range, 5 to 66 min). A typical serum concentration disposition curve is shown in Figure 1. Neither Resimmune C_{max} nor $t_{1/2}$ values were related to response or toxicity in this small study.

Pretreatment concentrations of circulating antibodies were assayed in all 30 patients and ranged from 0.8 to 251 μg/mL with a mean of 22 μg/mL, most likely reflecting prior immunization with diphtheria toxoid in childhood (*Online Supplementary Table S4*). In the 27 patients who had Resimmune antibody titers measured after completion of the cycle, antibody titers increased in all except one patient. The mean pretreatment antibody titer for the 28 patient treatments with both pre- and post-antibody levels was 18 μg/mL (range, 0.8 to 251 μg/mL), while the mean post-treatment antibody titer was 925 μg/mL (1 to 5451 μg/mL). Neither the type nor the number of prior therapies was a determinant of the pretreatment antibody titer. Pretreatment antibody titer was weakly inversely related to C_{max} and strongly related to T-cell depletion with Pearson r= -0.4 (n=14) and Pearson r=0.81 (n=20), respectively, yielding a two-tailed P=0.16 for the correlation with C_{max} and a two-tailed P<0.0001 for the correlation with T-cell depletion. Neither pretreatment nor post-treatment antibody titer values were related to response or toxicity in this small study.

Mean circulating $CD3^+$ T cells were assayed in 20 patients on day 0 and day 4 or 5 (*Online Supplementary Table S5*). The percentage of T cells compared to that at baseline is shown and ranged from <0.1% to 69% with a mean of

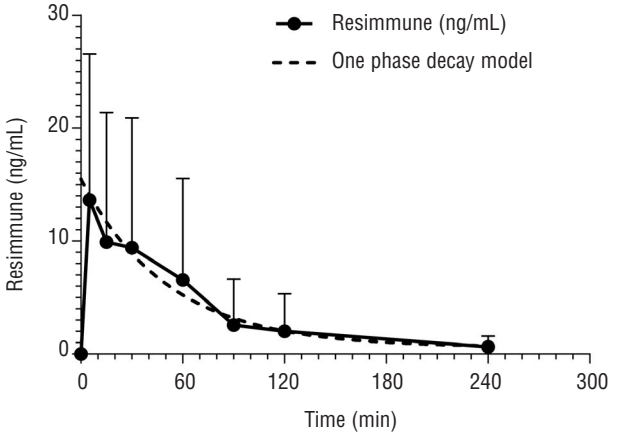

Figure 1. A serum concentration disposition curve of Resimmune. Each time point was calculated from results of patients #1-6, 9, 10, 16 and 18. Error bars indicate standard deviation. A one phase decay model was used to calculate the serum half-life of Resimmune (35.69 min).

Table 3. Pharmacokinetics studies

Dose level (μg/kg)	N.	Cmax (ng/mL) median/range	Half-life (min) median/range	AUC(ng*min/mL) median/range
2.5	6	20 (3-41)	43 (41-66)	1300 (190-3300)
5	5	2 (0-3)	44 (5-44)	115 (28-115)
7.5	2	2 (0-2)	39	101
11.25	2	16 (0-16)	12	126

Table 4. Clinical response summary*.

Subjects	N.	ORR (%)	CRR (%)	PRR (%)	Duration of response (months)
All	30	30	13	17	3,3,3+,6+,14,24+,60+,72+,72+
CTCL	25	36	16	20	3,3,3+,6+,14,24+,60+,72+,72+
Stage IB/IIB	17	47	18	29	3,3,3+,6+,14,24+,72+,72+
Stage III/IV	8	13	13	0	60+
mSWAT<50	17	53	24	29	3,3,3+,6+,14,24+,60+,72+,72+
mSWAT>50	8	0	0	0	---
Stage IB/IIB & mSWAT < 50	11	73	27	46	3,3,3+,6+,14,24+,72+,72+
Stage III/IV & mSWAT >50	3	0	0	0	---
Non-CTCL	5	0	0	0	---

**ORR, overall response rate; CRR, complete response rate; PRR, partial response rate.*

11%. There was a weak inverse relationship of pretreatment C_{max} with T-cell depletion with r= -0.4 (n=12) and a two-tailed *P*=0.2. However, T-cell depletion was not correlated with dose, response or toxicities.

Clinical response

Table 4 details the patients' and drug-dosing parameters related to response and response duration for each subject. Responses were seen only in CTCL patients. Among 25 patients with CTCL, we observed nine responses for a response rate of 36% (95% CI, 18%-57%). There were four complete responses (16% complete response rate, 95% CI, 5%-36%) lasting 72+, 72+, 60+ and 24+ months. There were five partial remissions with durations of 3, 3, 3+, 6+, and 14 months. The median response duration is 14 months (range, 3 to 72+ months). One patient was retreated and again had a partial response. A long time was required to convert from a partial response to a complete one (Figure 2). The extent of prior therapy, drug dose, drug C_{max}, circulating T-cell depletion, and pretreatment anti-diphtheria toxin antibody titers were not significant determinants for response or response duration. In contrast, the extent of disease at treatment, defined by mSWAT scores and stage, showed that patients with mSWAT<50 (n=17) or stage IB/IIB disease (n=17) or both (n=11) had higher likelihoods of response with nine (53%), eight (47%), and eight (73%) responses, respectively, compared with patients with mSWAT >50 (n=8) or III-stage IV disease (n=8) or both (n=3) of whom none, one and none, respectively, had responses (*P*=0.017 for mSWAT and *P*=0.165 for stage by the Fisher exact test). The improvement in a patient's skin lesions is shown in Figure 3.

Discussion

This study demonstrates that Resimmune, a recombinant immunotoxin targeting CD3ε, has robust activity in intermediate stage (IB or mSWAT <50) CTCL patients. The study is the first complete report of the phase 1 evaluation of Resimmune. Currently, CTCL patients have a large number of treatment options including skin-directed therapies, systemic therapies with cytotoxic chemotherapies, histone deacetylase inhibitors and rexinoids, and allogeneic stem cell transplants.[17-20] Although objective responses to initial treatments are common, most responders, except for allogeneic stem cell transplant recipients, develop recurrent disease within several months or years. Patients with stage IB and IIB disease cycle through numerous treatments and suffer the chronic toxicities, costs, physical inconvenience of multiple physician visits, and, eventually in a significant fraction, progressive disease and death.[18] In this setting, Resimmune offers a number of advantages. First, the treatment course is short, being just 4 days. Second, the side effect profile is moderately tolerable: transient VLS and rare immunocompromised host infections. VLS is mitigated by albumin infusions. Immunocompromised host viral infections were reversible with rituximab and/or antiviral medications. Third, some patients achieve durable remissions lasting years.

CTCL patients showed a high response rate to Resimmune. A direct comparison with denileukin diftitox cannot be made from the current study, both because of the sample sizes and the study designs. Nevertheless, in early stage patients, the activity of Resimmune appears to be at least comparable and perhaps improved. Explanations may include the higher density of CD3 receptors relative to interleukin-2 receptors.[22] Furthermore, Resimmune has greater affinity for its receptor and greater potency *in vitro*.[11,23] A randomized phase 2 trial would be necessary to address relative clinical benefits.

The onset of responses to Resimmune was gradual. Most patients showed maximal improvement in skin lesions only after several months. Such behavior has been described recently for immune checkpoint modulators including ipilimumab and pembrulizumab.[24] The median response duration after a single cycle of treatment was remarkable at longer than 2 years. Again, immune modulators have produced similarly durable responses in advanced melanoma and renal cell carcinoma. This effect was also seen, albeit infrequently, for other diphtheria fusion proteins including

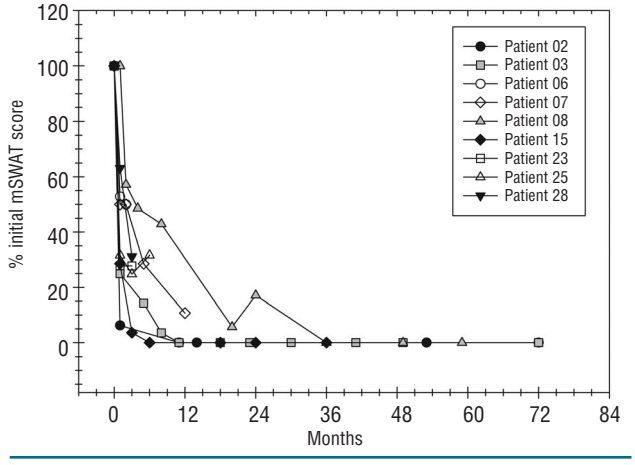

Figure 2. Reduction of mSWAT scores over time. Changes of % initial mSWAT score for patients #2, 3, 6, 7, 8, 15, 23, 25 and 28.

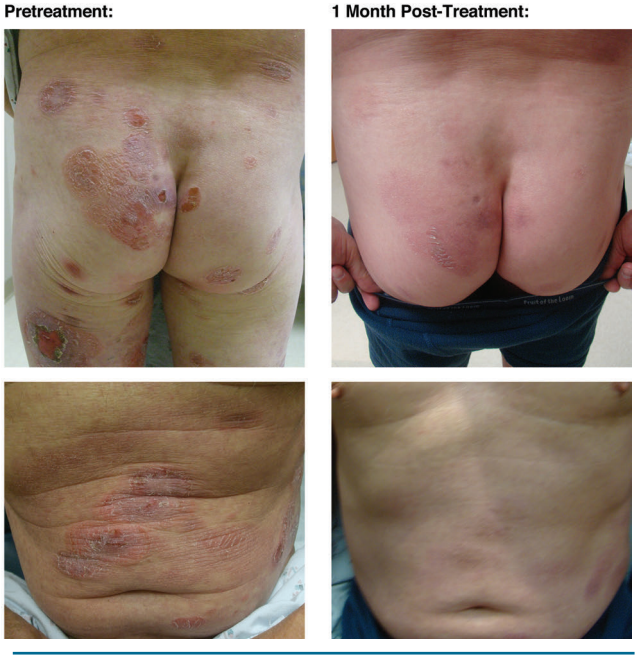

Figure 3. Photographs of patient #15 before treatment and 1 month after treatment.

denileukin diftitox and SL-401.[19,25] In tissue culture studies, we observed diphtheria immunotoxin-induced necroptosis with release of HMGB-1.[26] These findings are consistent with immunogenic cell death. The lymphoma cell debris alerts the innate immune system. In xenograft models, T-cell-directed immunotoxins also appear to alter the immune suppression of the microenvironment.[27] Lymphodepletion enhanced anti-tumor immunity in both animal models and patients.[28] Thus, Resimmune may inhibit lymphoma growth by several immune mechanisms in addition to cell cytotoxicity.

Responses were limited to patients with stage IB/IIB CTCL. The lack of activity in stage III/IV CTCL may reflect the lower levels of CD3ε or T-cell receptor in more advanced disease.[29] CTCL lymphocytes may lose dependence on antigen-driven T-cell receptor signaling.[30] Furthermore, there is evidence for different methylation patterns and gene expression profiles in higher stage CTCL.[31,32]

Although dramatic reductions in T-cell counts were observed by day 4 or 5 in 70% of evaluable patients, only seven patients had EBV and CMV viremia. Only a single patient (#29) had a clinical EBV infection with liver failure, renal failure and metabolic acidosis. All the other patients had monitoring of EBV and CMV by polymerase chain reaction and responded to rituximab for EBV and gancyclovir for CMV without clinical consequences. The relatively mild clinical course after profound suppression of circulating mature T cells was likely due to homeostatic repopulation as we documented in an earlier report.[33] The Resimmune-mediated 2-week recovery of memory T-cell populations was much faster than observed with alemtuzumab, visilizumab or fludarabine.

VLS was associated with hypoalbuminemia, edema, fatigue, and hypotension. The syndrome was observed after 3 to 4 days of treatment and was generally mild to moderate. In two patients with a prior history of heart failure (patients #10 and #18), irreversible congestive heart failure occurred. Consequently, patients with a history of heart disease were ineligible for the study. The severity of VLS-related adverse events was reduced by administration of parenteral albumin and diuretics (e.g., furosemide). Clinical VLS has been reported with other fusion proteins incorporating diphtheria toxin or Pseudomonas exotoxin fragments.[34,35] Tissue culture and animal experiments implicated non-specific immunotoxin uptake by vascular endothelium as the probable mechanism for VLS.[36] Once internalized, the catalytic proteins induce endothelial cell shrinkage and apoptosis and capillary permeability.[37]

Resimmune's pharmacokinetic and immunological characteristics in CTCL patients were similar to those of denileukin diftitox.[35] A lack of correlation of pharmacokinetic parameters with toxicity or response may be due to the extreme potency of Resimmune, which is cytotoxic at picomolar concentrations. A similar lack of association between pharmacokinetic parameters and immune response was seen with other diphtheria toxin fusion proteins.[25,35,38,39] However, in the subset of patients with high pre-treatment anti-diphtheria toxin titers (>32 μg/mL), circulating drug levels were not measurable, possibly due to rapid clearance. All study patients had pretreatment antibodies to diphtheria toxin (antibody titers >0.2 μg/mL), most likely due to prior immunization with diphtheria toxoid. These results are similar to the findings of pretreatment anti-diphtheria toxin antibodies in 89% of patients with acute myeloid leukemia.[38,39] Low (0.2 – 2.4 μg/mL) and intermediate (2.5 – 50 μg/mL) antibody titers were present in 13% and 76% of pretreatment T-cell lymphoma patients, respectively, *versus* 67% and 22% of patients with acute myeloid leukemia, respectively.[39] The higher levels of anti-diphtheria toxin antibodies in our study may reflect less extensive prior chemotherapy or better immunological status of the CTCL patients compared to the patients with acute myeloid leukemia. Alternatively, the anti- diphtheria toxin antibody titers may reflect different immunization histories. Antibody titers correlated with degree of T-cell depletion but not with pharmacokinetic behavior, antitumor activity or toxicities.

There are other opportunities to target CD3, which may modify human disease processes. Resimmune-mediated T-cell depletion may selectively deplete T regulatory cells in the tumor microenvironment. Denileukin diftitox depleted $CD4^{+}CD25^{HI}Foxp3^{+}$ regulatory T cells and expanded melanoma-specific $CD8^{+}$ T cells in mice bearing human melanoma xenografts.[27] Chesney and colleagues then treated 60 stage IV melanoma patients with denileukin diftitox and observed partial responses and stable disease in 17% and 5%, respectively.[40] Based on clinical Resimmune-induced modifications of T-cell subsets,[33] Chesney recently began a clinical trial of Resimmune plus radiation therapy in stage IV melanoma patients (NCT01888081). The hypothesis is that Resimmune will overcome the T regulatory cell immune checkpoint barrier. Another application may be in autoimmune disorders. Miniature swine given haploidentical stem cell transplants with low dose total body irradiation, anti-swine CD3 immunotoxin, and a short course of cyclosporine engrafted with no significant graft-*versus*-host disease.[41] Thus, Resimmune may be useful in HLA-mismatched allogeneic stem cell transplants for graft-*versus*-host disease prophylaxis or treatment. Streptozotocin-induced diabetic rhesus macaques given allogeneic islets combined with anti-monkey CD3 immunotoxin and deoxyspergualin showed durable restoration of islet function with chronic immunosuppressive therapy.[42] Similarly, miniature swine given musculoskeletal tissue allografts achieved tolerance with anti-swine CD3 immunotoxin plus a short course of cyclosporine.[43] Hence, Resimmune may be useful for inducing tolerance in solid organ transplants. Other mature T-cell malignancies with high levels of surface CD3 expression may be suitable targets for Resimmune, including T-cell large granular lymphocytosis, intestinal T-cell lymphoma and hepatosplenic T-cell lymphoma. Because Resimmune has distinct and non-overlapping cytotoxic mechanisms and toxicities compared with other CTCL therapeutics, combinations may yield an improved therapeutic index as observed in xenograft models with other immunotoxins and cytotoxic drugs.[44]

In summary, this phase I study supports the advancement of Resimmune into pivotal phase 2 trials in CTCL and other mature T-cell neoplasms to firmly establish its niche in the management of these diseases.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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