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

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Key Points: Resimmune was moderately tolerated and a single course of treatment produced a high rate of durable objective responses in stage IB-IIB with mSWAT<50 cutaneous T cell lymphoma patients.

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. 25 cutaneous T cell lymphoma (CTCL) patients, 3 peripheral T cell lymphoma patients, and 1 T-cell large granular lymphocytic leukemia patient and 1 T-cell prolymphocytic leukemia patient received 15 minute i.v. infusions of Resimmune 2.5 - 11.25μg/kg in an inter-patient dose escalation trial. The most common adverse events were fever, chills, hypotension, edema, hypoalbuminemia, hypophosphatemia, and transaminasemia. Among 25 CTCL patients, there were nine responses for a response rate of 36% (95% CI, 18%-57%) including four complete remissions (16%, 95% CI, 5%-36%). CR durations were 72+, 72+, 60+ and 38+ months. There were five PRs lasting 3, 3, 3+, 6+ and 14 months. Patients with a modified skin weighted assessment tool (mSWAT) score <50, patients with stage IB/IIB, or patients with both had 9/17 (53%), 8/17 (47%), and 8/11 (73%) responses, respectively. Further studies of Resimmune in low tumor burden IB-IIB CTCL patients 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 U.S.[1,2] There are multiple approved topical and systemic therapies including topical nitrogen mustard, oral bexarotene, romidepsin, and vorinostat, alemtuzumab, extracorporeal photopheresis, and allogeneic stem cell transplantation.[3-5] Most of the treatments require chronic or 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 transplant may provide long-term remissions, most therapies yield responses lasting months. Overall, CTCL has a long clinical course with relentless progression for many patients over months to years with an estimated median survival of 3-5 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). Partial remissions (PRs) were observed in a third of patients for several of the agents.

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, nodes and spleen of transgenic mice. Resimmune bound only splenic lymphocytes among eighteen normal human tissues, and mice, rats and monkeys given total doses of >200μg/kg over 4 days showed only transient transaminasemia without histopathologic tissue injury or clinical signs or symptoms.[12] Based on these results, we achieved FDA approval for testing in T cell neoplasm patients (BB IND#100712). The starting dose (2.5μg/kg x8) was 1/10 the MTD observed in monkeys.[12] This report describes the results of this study.

Methods

The Resimmune study was a single-arm, multi-center 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 in 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 11.25μg/kg intravenously twice daily for 4 days.

Eligibility and diagnosis

Patients with CD3+ T cell malignancies diagnosed by morphologic, histochemical, and cell surface criteria and having failed a systemic therapy were eligible for the study.

Treatment

Resimmune was given as 2.5, 5, 7.5, or 11.25μg/kg twice daily (4-6 hours apart) for 4 consecutive days through a free flowing IV over 15 minutes. In the dose escalation portion of the study, cohorts of 3 patients were treated at each dose level unless dose-limitingt toxicity (DLT) was observed in one patient in which case the cohort was expanded to six patients. Once 2 patients at a dose level experienced DLT, the next lower dose level was the MTD. In the expansion cohort, 13 additional CTCL patients were treated at the MTD of 7.5μg/kg dose.

Toxicity and response evaluation

Toxicities were determined before treatment and daily for four days and then on days 10, 23, 37, and at follow-up visits by history, physical exams, CBC with differential, serum chemistries. ECG was done before treatment and on day 1 and 4. Blood EBV and CMV PCR titers were obtained before treatment and on day 4, 10, 16, 23, and 37. 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 mSWAT score.[13] Complete response (CR) required an mSWAT score of 0, normal liver and spleen size, absence of pathologic adenopathy by exam and CT scan, and normal bone marrow biopsy and aspirate. PR must show a 50% reduction in mSWAT and with no new skin lesions and no pathologic involvement of nodes, marrow or visceral organs. Progressive disease (PD) is associated with a 25% increase in mSWAT or new non-skin disease. Stable disease (SD) is absence of CR, PR, or PD.

Pharmacology/immune response/flow cytometry

Resimmune concentrations in serum samples were measured by a bioassay using Jurkat cells.[14,15] Cmax, serum half-life and AUC (Area Under the Curve) were determined. 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 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 vs any or none and mild vs moderate to severe. The rates of toxicity, overall response, and CR, as well as their 95% confidence intervals were estimated using an exact binomial method. The mean and standard deviation values of the pharmacokinetic (PK) parameters including maximum concentration (C_{max}) and half-life ($t_{1/2}$) were reported.

Results

Patients

Thirty patients were treated with 31 courses of Resimmune; one patient received a second course of treatment six months on disease recurrence. All 30 patients were evaluable for safety, whereas 26 patients were evaluable for objective response. Twenty-six patients received all 8 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 <8 doses during the treatment period were hypotension and hypoalbuminemia with or without hypoxia or congestive heart failure.

Relevant patient demographic and prior treatment information is detailed in Table 1 and S1. There were 18 females and 12 males; the median age was 57 years with a range of 20 to 84 years. The patients had received an average of 3 prior therapies including 4 patients with a single prior regimen and two patients with multiple modalities including allogeneic stem cell transplants. 17 patients had CTCL stage IB-IIB; eight patients had CTCL stage III-IV; 3 patients had PTCL; and 1 patient had T-LGL and 1 patient had T-PLL. The study was subsequently modified to exclude patients with a prior history of heart disease and recent alemtuzumab therapy.

Toxicities

Adverse events (AEs) attributed to drug treatment at the 2.5 – 11.5μg/kg dose levels as listed in Table 2 and Table S2. There were three Grade 4/5 drug-related toxicities. Patient #10 treated at 5μg/kg developed severe congestive heart failure and vascular leak syndrome after 5 doses and expired on day 11. He had a history of previous congestive heart failure and cardiomegaly. Patient #18 treated at 11.25μg/kg also developed severe congestive heart failure and vascular leak syndrome after 6 doses and expired on day 9. She had a history of pulmonary hypertension and right ventricular dilatation. Patient #28 was treated at 7.5μg/kg for 8 doses and had EBV reactivation and EBV-induced liver and renal failure and died on day 29. He had a course of alemtuzumab four months before and had 211/μL CD3+CD4+ T cells prior to Resimmune. After these AEs, the protocol was modified to exclude patients with a history of heart disease or recent alemtuzumab. Additional Grade 3 AEs included six patients with EBV and/or CMV reactivation, two patients with hypophosphatemia, and two patients with 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 7.5μg/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 vascular leak syndrome or hypoalbuminemia, and two patients with history of heart disease had grade 4-5 vascular leak syndrome. 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 was not predictive of VLS. Steroids were not used for VLS management.

CMV and/or EBV reactivation based on blood PCR assays occurred in seven patients. In six patients, there were no associated symptoms, and the patients responded to gancyclovir orally and/or rituximab intravenously. One patient with pretreatment lymphopenia post-alemtuzumab developed EBV viremia on day 24. He refused rituximab and expired with multi-organ failure five days later.

Six patients experienced isolated elevation of hepatic transminases without hyperbilirubinemia. Four patients 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 on treatment including two patients with hypophosphatemia and one patient each with hypocalcemia and hypomagnesemia. Each patient responded quickly to electrolyte replacement.

14 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 H-1 and H2-histamine antagonists. Symptoms included fever and/or chills. Three patients had transient hypotension, and one patient hypoxemia. All these reactions resolved rapidly after administration of fluids or oxygen, respectively.

Pharmacologic, immunologic and flow cytometry studies

Serum samples for pharmacokinetic studies were collected on day 1 and 2 for 14 patients and for immune response measurements were done on day 1 for all 30 patients and day 10 - 30 for 18 patients. Blood flow cytometry assays of circulating T cells were done on day 0 and day 4 or 5 for 20 patients. The results of relevant pharmacologic and immunologic and circulating cell populations are shown in Table 3 and Tables S3-S5. Cmax values averaged 7.9 ng/mL with a range from 0 to 41ng/mL, after treatment on day 1. Absent detectable drug levels in PK samples were seen in samples from patients 8, 11, 13, 14, and 17. The clearance of Resimmune generally fit a mono-exponential model. Drug clearance was highly variable with $t_{1/2}$ values averaging 39 min with a range of 5 to 66 min. A typical serum concentration disposition curve is shown in Figure 1. Neither Resimmune Cmax nor $t_{1/2}$ values were related to response or toxicity in this small study.

Pretreatment concentrations of circulating antibodies was tested 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 (Table S4). In the 27 patients who had Resimmune antibody titers measured after completion of the cycle, antibody titers increased in all except 1 patient. The mean pretreatment antibody titer for the 28 patient treatments with both pre and post antibody level was 18μg/mL with a range of 0.8 to 251μg/mL, and the mean posttreatment antibody titer was 925μg/mL with a range of 1 to 5451μg/mL. Type or number of prior therapies was not a determinant in the pretreatment antibody titer. Pretreatment antibody titer

was weakly inversely related to Cmax and strongly related to T cell depletion with Pearson r= -0.4 for n=14 and Pearson r=0.81 and n=20, respectively, yielding a p=0.16 two-tailed for correlation with Cmax and p<0.0001 two-tailed for correlation with T cell depletion. Neither pretreatment nor post-treatment antibody titer values 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 (Table S5). The percentage T cell compared to baseline is shown and ranged from <0.1% to 69% with a mean of 11%. There was a weak inverse relationship of pretreatment Cmax to T cell depletion with r= -0.4 n=12 and p=0.2 two-tailed. However, there was no correlation of T cell depletion with dose, response or toxicities.

Clinical Response

Table 4 details the patient and drug dosing parameters related to response and response duration for each subject. Responses were seen only in CTCL patients. Among 25 CTCL patients, we observed nine responses for a response rate of 36% (95% CI, 18%-57%). There were four CRs (16% CR rate, 95% CI, 5%-36%) lasting 72+, 72+, 60+ and 24+ months. There were five PRs with durations of 3, 3, 3+, 6+, and 14 months. The median response duration is 14 months with a range of 3 to 72+ months. One patient was retreated and again had a PR. A long time was required to convert from a PR to a CR (Figure 2). The extent of prior therapy, drug dose, drug Cmax, 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 patients with mSWAT<50 or IB/IIB disease or both had higher likelihood of response with 9/17 responses (53%), 8/17 responses (47%), and 8/11 responses (73%), respectively, compared with patients with mSWAT > 50 or III-IV disease or both with 0/8 responses, 1/8 responses, and 0/3 responses, respectively (p = 0.017 for mSWAT and p=0.165 for stage by Fisher's exact test). Diminishment of patient 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--four days. Second, the side effect profile is moderately tolerable—transient vascular leak syndrome (VLS) and rare immunocompromised host infections. VLS is mitigated by albumin infusions. Immunocompromised host viral infections were reversible with rituximab an/or antiviral medications. Third, a fraction of patients achieve durable remissions lasting years.

Resimmune showed a high response rate in CTCL patients. Direct comparison with denileukin diftitox cannot be made from the current study, both due to sample sizes and study designs. Nevertheless, in early stage patients, the Resimmune activity appears at least comparable and perhaps improved. Explanations may include the higher density of CD3 receptors relative to interleukin-2 receptors (IL2Rs).[22] Further, Resimmune has higher affinity for its receptor and greater potency *in vitro*.[11,23] A randomized phase 2 trial would be necessary to address relative clinical benefit.

Onset of responses 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] Median response duration after a single cycle was remarkable at greater than two years. Again, immune modulators have produced similar durable responses in advanced melanoma and renal cell carcinoma. This phenomenon was also seen, albeit infrequently, for other diphtheria fusion proteins including 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 both in animal models and patients.[28] Thus, Resimmune may inhibit lymphoma growth by several immune mechanisms in addition to cell cytotoxicity.

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

Although dramatic reductions in T cells were observed by day 4/5 in 70% of evaluable patients, only seven patients displayed 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 PCR 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 two week recovery of memory T cell populations is much faster than observed with alemtuzumab, visilizumab or fludarabine.

VLS was associated with hypoalbuminemia, edema, fatigue, and hypotension. VLS was observed after three to four days of treatment and was generally mild to moderate. In two patients with 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 (eg, furosemide). Clinical VLS has been reported with other fusion proteins incorporating DT or Pseudomonas exotoxin fragments.[34,35] Tissue culture and animal experiments implicated nonspecific immunotoxin uptake by vascular endothelium as the probable mechanism for the VLS.[36] Once internalized, the catalytic proteins induce endothelial cell shrinkage and apoptosis and capillary permeability.[37]

Resimmune's PK and immunologic characteristics in CTCL patients were similar to those of denileukin diftitox.[35] Lack of correlation of PK parameters with toxicity or response may be due to the extreme potency of Resimmune, which is cytotoxic at picomolar concentrations. Similar dissociation of PK and immune response was seen with other DT fusion proteins.[25,35,38,39] However, in the subset of patients with high pre-treatment anti-DT titers (>32μg/mL), circulating drug levels were not measurable possibly due to rapid clearance. All study patients had pretreatment antibodies to DT (antibody titers >0.2μg/mL), most likely due to prior immunization with diphtheria toxoid. These results are similar to the findings of pretreatment anti-DT antibodies in 89% of acute myeloid leukemia (AML) patients.[38,39] Low (0.2 – 2.4μg/mL) and intermediate (2.5 – 50μg/mL) antibody titers were present in 13% and 76% of pretreatment T cell lymphoma patients, respectively, versus 67% and 22% of AML patients, respectively.[39] The higher levels of anti-DT antibodies in our study may reflect less extensive prior chemotherapy or better immunological status of the CTCL patients compared to the AML patients. Alternatively, the anti-DT antibody titers may reflect differing immunization histories. Antibody titers correlated with degree of T cell depletion but not with PK behavior, antitumor activity nor toxicities.

There are other opportunities to target CD3 that may modify human disease processes. Resimmune 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 PR and SD in 17% and 5%, respectively.[40] Based on clinical Resimmune-induced T cell subset modifications,[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 (GVHD).[41] Thus, Resimmune may be useful in HLA-mismatched allogeneic stem cell transplants for GVHD 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 give musculoskeletal tissue allografts achieved tolerance with anti-swine CD3 immunotoxin plus a short course of cyclosporine.[43] Hence, Resimmune may be useful in solid organ transplant tolerance induction. 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 nonoverlapping cytotoxic mechanism 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 morbid diseases.

Authorship and Disclosures

AEF was the principal investigator and takes primary responsibility for the paper; AEF, FMF, and MD recruited and treated the patients on the study; JHW performed the laboratory work for this study; CA did the statistical analyses; PHN, DMN coordinated the research; AEF, JHW, CA, FMF, MD, PHN and DMN wrote the paper. AEF, FMF, MD, and JHW had research support from Angimmune, LLC, and JHW, DMN and PHN have financial interests in Angimmune, LLC.

References

1. Wong HK, Mishra A, Hake T, Porcu P. Evolving insights in the pathogenesis and therapy of cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome). Br J Haematol. 2011;155(2):150-166.
2. Imam MH, Shenoy PJ, Flowers CR, Phillips A, Lechowicz MJ. Incidence and survival patterns of cutaneous T-cell lymphomas in the United States. Leuk Lymphoma. 2013;54(4):752-759.
3. Jawed SI, Myskowski PL, Horwitz S, Moskowitz A, Querfeld C. Primary cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome) Part I. Diagnosis: Clinical and histopathologic features and new molecular and biologic markers. J Amer Acad Dermatol. 2014;70(2):205.e1-205.e16.
4. Talpur R, Singh L, Daulat S, et al. Long-term outcomes of 1,263 patients with mycosis fungoides and Sezary syndrome from 1982 to 2009. Clin Cancer Res. 2012;18(18):5051-5060.
5. Jawed SI, Myskowski PL, Horwitz S, Moskowitz A, Querfeld C. Primary cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome) Part II. Prognosis, management, and future directions. J Amer Acad Dermatol. 2014;70(2):223.e1-223.e17.
6. FitzGerald DJ, Wayne AS, Kreitman RJ, Pastan I. Treatment of hematologic malignancies with immunotoxins and antibody-drug conjugates. Cancer Res. 2011; 71(20): 6300-6309.
7. Thompson J, Stavrou S, Weetall M, et al. Improved binding of a bivalent single-chain immunotoxin results in increased efficacy for in vivo T-cell depletion. Protein Eng. 2001;14(12):1035-1041.
8. Arnett KL, Harrison SC, Wiley DC. Crystal structure of a human CD3-ε/δ dimer in complex with a UCHT1 single-chain antibody fragment. Proc Natl Acad Sci USA. 2004;101(46):16268-16273.
9. Izban KF, His ED, Alkan S. Immunohistochemical analysis of mycosis fungoides on paraffin-embedded tissue sections. Mod Pathol. 1998;11(10):978-982.
10. Hexham JM, Dudas D, Hugo R, et al. Influence of relative binding affinity on efficacy in a panel of anti-CD3 scFv immunotoxins. Mol Immunol. 2001;38(5):397-408.
11. Woo JH, Liu JS, Kang SH, et al. GMP production and characterization of the bivalent anti-human T cell immunotoxin, A-dmDT390-bisFv(UCHT1) for phase I/II clinical trials. Protein Expr Purif. 2008;58(1):1-11.
12. Woo JH, Bour SH, Dang T, et al. Preclinical studies in rats and squirrel monkeys for safety evaluation of the bivalent anti-human T cell immunotoxin, A-dmDT390-bisFv(UCHT1). Cancer Immunol Immunother. 2008;57(8):1225-1239.
13. Scarisbrick JJ, Kim YH, Whittaker SJ, et al. Prognostic factors, prognostic indices and staging in mycosis fungoides and Sezary syndrome: where are we now? Br J Dermatol. 2014; 170(6): 1226-1236.

14. Neville DM, Srinivasachar K, Stone R, Scharff J. Enhancement of immunotoxin efficacy by acid-cleavable cross-linking agents utilizing diphtheria toxin and toxin mutants. J Biol Chem 1989;264(25):14653-14661.
15. Thompson J, Hu H, Scharff J, Neville DM. An anti-CD3 single-chain immunotoxin with a truncated diphtheria toxin avoids inhibition by pre-existing antibodies in human blood. J Biol Chem 1995;270(47):28037-28041.
16. Woo JH, Lee Y, Neville DM, Frankel AE. Pharmacology of anti-CD3 Diphtheria immunotoxin in CD3 positive T cell lymphoma trials. Methods Mol. Biol. 2010;651:157-175.
17. Wilcox RA. Cutaneous T-cell lymphoma: 2014 update on diagnosis, risk-stratification, and management. Am J Hematol. 2014;89(8):837-851.
18. Jawed SI, Myskowski PL, Horwitz S, et al. Primary cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome) Part II. Prognosis, management, and future directions. J Am Acad Dermatol. 2014;70(2):223.e1-223.e17.
19. Duvic M, Geskin L, Prince HM. Duration of response in cutaneous T-cell lymphoma patients treated with denileukin diftitox: results from 3 phase III studies. Clin Lymphoma Myeloma Leuk. 2013;13(4):377-384.
20. Schmitz N, Wu HS, Glass B. Allogeneic transplantation in T-cell lymphomas. Semin Hematol. 2014;51(1):67-72.
21. Jokinen CH, Fromm JR, Argenyi ZB, et al. Flow cytometric evaluation of skin biopsies for mycosis fungoides. Am J Dermatopathol. 2011;33(5):483-491.
22. Gniadecki R. Lack of membrane expression of interleukin-2 receptor α chain (CD25) in mycosis fungoides: application of laser scanning cytometry for phenotyping of skin infiltrating lymphocytes. J Invest Dermatol. 2000;114(3):594-595.
23. Re GG, Waters C, Poisson L, et al. Interleukin 2 (IL-2) receptor expression and sensitivity to diphtheria fusion toxin DAB389IL-2 in cultured hematopoietic cells. Cancer Res. 1996;56(11):2590-2595.
24. Hamid O, Robert C, Daud A, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. New Engl J Med. 2013;369(2):134-144.
25. Frankel AE, Woo JH, Ahn C, et al. Activity of SL-401, a targeted therapy directed to interleukin-3 receptor, in blastic plasmacytoid dendritic cell neoplasm patients. Blood. 2014;124(3):385-392.
26. Thorburn J, Horita H, Redzic J, et al. Autophagy regulates selective HMGB1 release in tumor cells that are destined to die. Cell Death Differ. 2009;16(1):175-183.
27. Jones E, Dahm-Vicker M, Simon AK, et al. Depletion of CD25+ regulatory cells results in suppression of melanoma growth and induction of autoreactivity in mice. Cancer Immun. 2002;2:1-12.
28. Klebanoff CA, Khong HT, Antony PA, et al. Sinks, supressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. Trends Immunol. 2005;26(2):111-117.
29. Johnson VE, Vonderheid EC, Hess AD, et al. Genetic markers associated with progression in early mycosis fungoides. J Eur Acad Dermatol Venereol. 2014;28(11):1431-1435.
30. Vaque JP, Gomez-Lopez G, Monsalvez V, et al. PLCG1 mutations in cutaneous T-cell lymphomas. Blood. 2014;123(13):2034-2043.
31. Ferrara G, Pancione M, Votino C, et al. A specific DNA methylation profile correlates with a high risk of disease progression in stage I classical (Alibert-Bazin type) mycosis fungoides. Br J Dermatol. 2014;170(6):1266-1275.

32. Litvinov IV, Jones DA, Sasseville D, Kupper TS. Transcriptional profiles predict disease outcome in patients with cutaneous T-cell lymphoma. Clin Cancer Res. 2010;16(7):2106-2114.
33. Frankel AE, Zuckero SL, Mankin AA, et al. Anti-CD3 recombinant diphtheria immunotoxin therapy of cutaneous T cell lymphoma. Curr Drug Targets. 2009;10(2):104-109.
34. Hassan R, Bullock S, Premkumar A, et al. Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bolus I.V. infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. Clin Cancer Res. 2007;13(17):5144-5149.
35. Olsen E, Duvic M, Frankel AE, et al. Pivotal phase III trial of two dose levels of denileukin diftitoxfor the treatment of cutaneous T-cell lymphoma. J Clin Oncol. 2001;19(2):376-388.
36. Lindstrom AL, Erlandsen SL, Kersey JH, Pennell CA. An in vitro model for toxin-mediated vascular leak syndrome: ricin toxin A chain increases the permeability of human endothelial cell monolayers. Blood. 1997;90(6):2323-2334.
37. Baluna R, Vitetta ES. An in vivo model to study immunotoxin-induced vascular leak in human tissue. J Immunother. 1999;22(1):41-47.
38. Frankel AE, Liu JS, Rizzieri D, Hogge D. Phase 1 clinical study of diphtheria toxin-interleukin 3 fusion protein in patients with acute myeloid leukemia and myelodysplasia. Leuk Lymphoma. 2008;49(3):543-553.
39. Frankel AE, Powell BL, Hall PD, et al. Phase 1 trial of a novel diphtheria toxin/granulocyte macrophage colony-stimulating factor fusion protein (DT388GMCSF) for refractory or relapsed acute myeloid leukemia. Clin Cancer Res. 2002;8(5):1004-1013.
40. Telang S, Rasku MA, Clem AL, et al. Phase II trial of the regulatory T cell-depleting agent, denileukin diftitox, in patiens with unresectable stage IV melanoma. BMC Cancer. 2011;11:515.
41. Cina RA, Wikiel KJ, Lee PW, et al. Stable multilineage chimerism without graft versus host disease following nonmyeloablative haploidentical hematopoietic cell transplantation. Transplantation. 2006;81(12):1677-1685.
42. Contreras JL, Jenkins S, Eckhoff DE, et al. Stable alpha- and beta-islet cell function after tolerance induction to pancreatic islet allografts in diabetic primates. Am J Transplant. 2003;3(2):128-138.
43. Hettiaratchy S, Melendy E, Randolph MA, et al. Tolerance to composite tissue allografts across a major histocompatibility barrier in miniature swine. Transplantation. 2004;77(4):514-521.
44. Hogge DE, Feuring-Buske M, Gerhard B, et al. The efficacy of diphtheria-growth factor fusion proteins is enhanced by co-administration of cytosine arabinoside in an immunodeficient mouse model of human acute myeloid leukemia. Leuk Res. 2004;28(11):1221-1226.

Table 1. Patients and disease characteristics

Characteristics	Number of subjects (N=30)
Age median (range)	57 (20-84)
Gender (male/female)	12/18
Race	
Caucasians	19
Black	10
Hispanic	1
Disease	
CTCL	25
Stage IB-IIB	17
Stage III-IV	8
PTCL	3
PLL	1
LGL	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

Table 2. Treatment detail and AEs

Cohort	Dose (μg/kg)	Doses received	Total dose in μg	N	Drug-related AEs (CTCAEv4.03 toxicity grade)	DLT
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 SVT (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 SVT (n=1) Grade 4 hypotension (n=1) Grade 3 uremia (n=1)	1

Table 3. Pharmacokinetics studies

Dose level (ug/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*

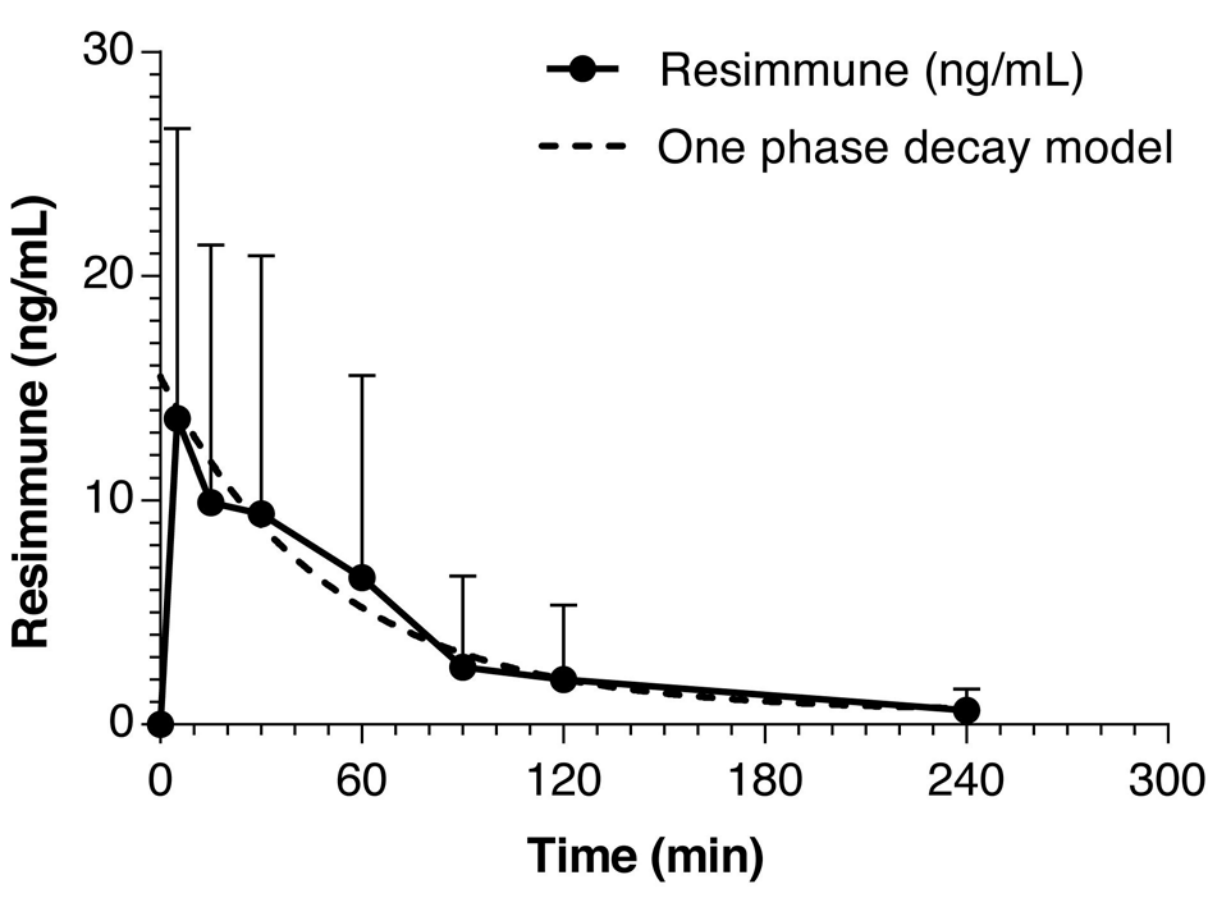
Subjects	N	ORR (%)	CRR (%)	PRR (%)	Length of response (mos)
All	30	30	13	17	3,3,3+,6+,14,24+,60+,72+,72+
CTCL	25	36	16	20	3,3,3+,6+,14,24+,60+,72+,72+
Stage IB/IIB	17	47	18	29	3,3,3+,6+,14,24+,72+,72+
Stage III/IV	8	13	13	0	60+
mSWAT<50	17	53	24	29	3,3,3+,6+,14,24+,60+,72+,72+
mSWAT>50	8	0	0	0	---
Stage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

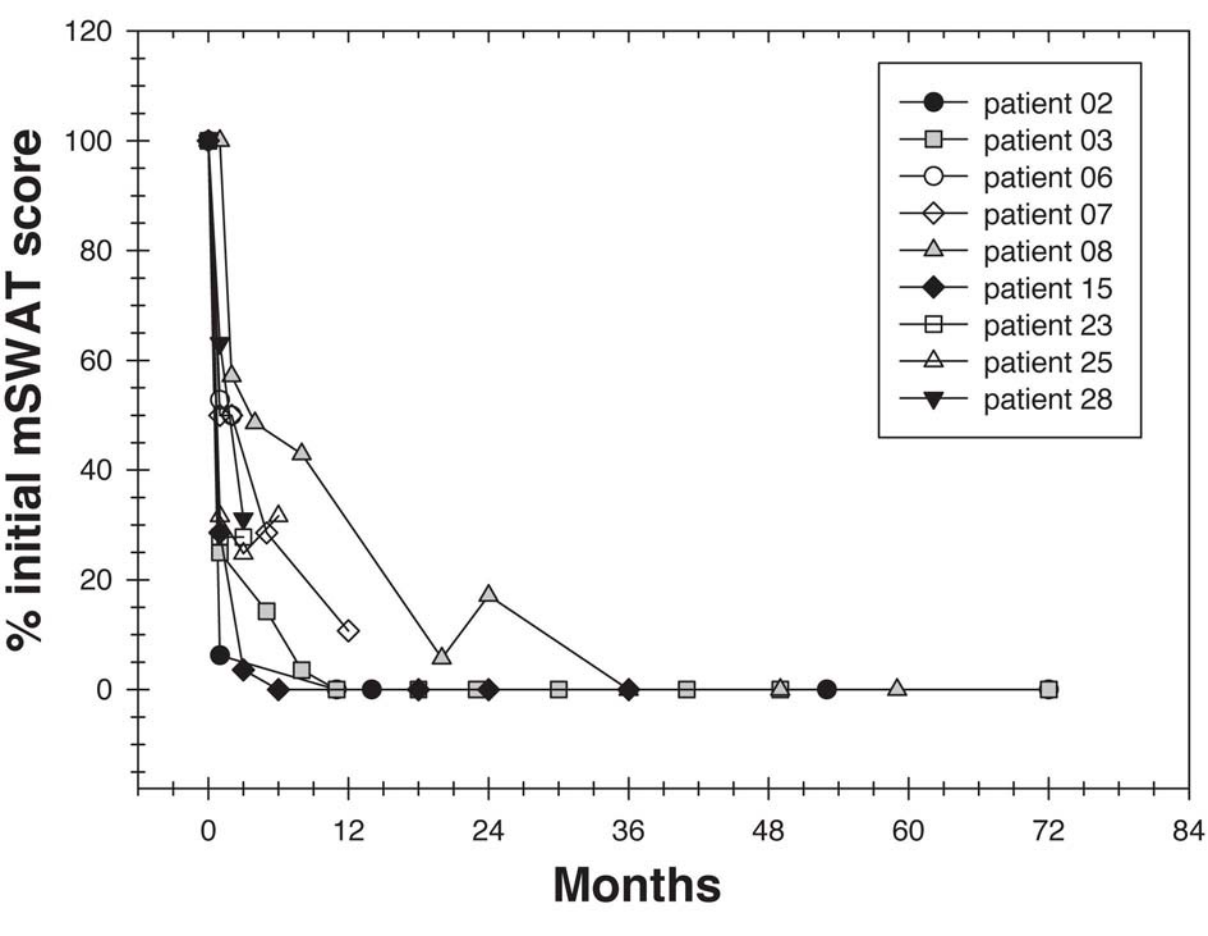
Figure Legends

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. One phase decay model was used to calculate a serum half-life of Resimmune (35.69 min).

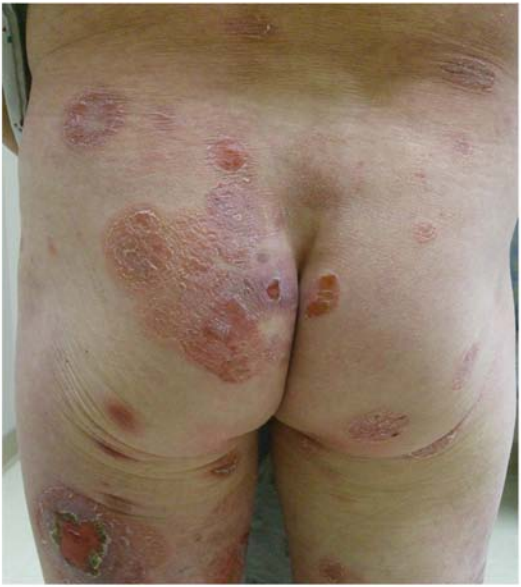
Figure 2. Reduction of mSWAT scores over time. Changes of % initial mSWAT score for patients #2, 3, 6, 7, 8, 15, 23, 25 and 28.

Figure 3. Photographs of patient #15 pretreatment and one month post-treatment.

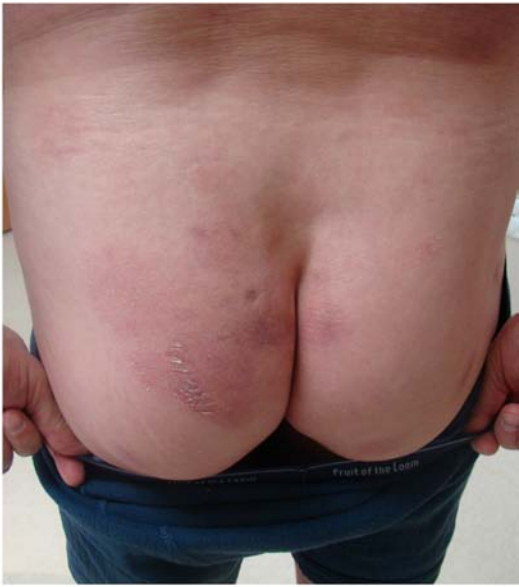

Resimmune (ng/mL)
One phase decay model
Resimmune (ng/mL)
30
20
10
0
0
60
120
180
240
300
Time (min)

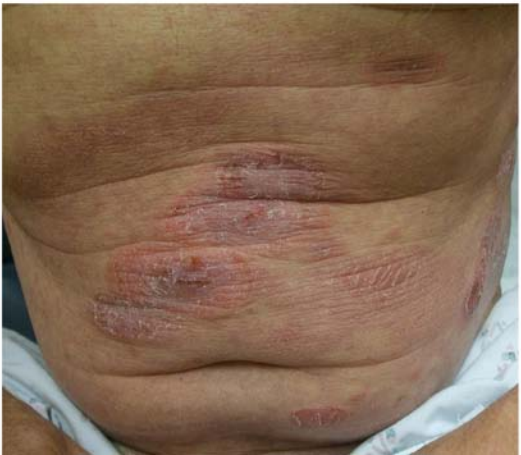

% initial mSWAT score
120
100
80
60
40
20
0
0
12
24
36
48
60
72
84
Months
patient 02
patient 03
patient 06
patient 07
patient 08
patient 15
patient 23
patient 25
patient 28

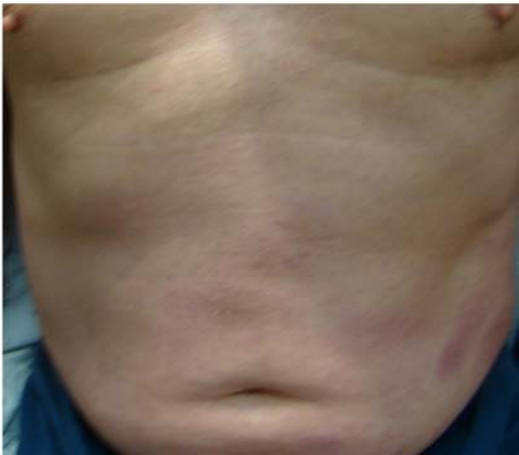
Pretreatment:



1 Month Post-Treatment:







Supplemental Material

Administration-- The study was performed under the sponsorship of Angimmune, LLC, registered in clinical trials.gov as NCT00611208, and approved by Institutional Review Boards at the participating institutions. In the dose escalation phase of the study, cohorts of new patients were treated with a single course of Resimmune as 15-minute infusions at doses ranging from 2.5 to 11.25μg/kg intravenously twice daily for 4 days. There was an expansion cohort at the maximal tolerated dose (MTD) in patients with stage IB-IIB CTCL and modified skin weighted assessment tool (mSWAT) scores of <50. In the expansion phase of the study, 13 patients received a single course at the 7.5μg/kg dose level.

Patient eligibility-- Patients with CD3+ T cell malignancies diagnosed by morphologic, histochemical, and cell surface criteria and having failed a systemic therapy were eligible for the dose-escalation portion of the study. Patients had to have CTCL stage IB/IIB with mSWAT <50 and have failed a systemic treatment for the expansion cohort. Patients had to have an ECOG performance status of <2 and give informed consent. Other eligibility requirements included the following: bilirubin <1.5mg/dL, transaminases <2.5x upper limit of normal, creatinine <2.0mg/dL, albumin >3g/dL, cardiac ejection fraction >50%, and willingness to use an approved form of birth control while on study. Patients with serious concurrent medical problems, active CNS leukemia, preexisting cardiovascular disease, cirrhosis with Child-Pugh score of Class B or C, and prior treatment with alemtuzumab were excluded.

Patient treatment-- Patients were treated at the University of Texas Southwestern Health Science Center, Baylor Scott & White Health Medical Center, the University of Texas M.D. Anderson Cancer Center, or Yale University Medical Center. Premedications administered prior to each dose of Resimmune included acetaminophen 650 mg orally, diphenhydramine 50 mg orally, ranitidine 150 mg orally and, optionally, 100 mg hydrocortisone intravenously. One liter 5% dextrose/0.45% NaCl intravenously was given daily for four days. Prophylactic acyclovir 400 mg orally twice daily and trimethoprim/sulfamethoxazole 800mg/160mg orally three times per week were given for two weeks. Resimmune was given as 2.5, 5, 7.5, or 11.25μg/kg twice daily (4-6 hours apart) for 4 consecutive days through a free flowing IV over 15 minutes. Doses on day 2, 3, and 4 were only given in absence of grade 3 non-hematologic toxicity. After 8 weeks, patients with evidence of disease progression were eligible for retreatment once provided the anti-diphtheria toxin titer was <30μg/mL, they had recovered to less than grade 2 non-hematologic toxicity, and the blood non-malignant resting T cell number was ≥300/μL. In the dose escalation portion of the study, cohorts of 3 patients were treated at each dose level unless dose-limiting toxicity (DLT) was observed in one patient in which case the cohort was expanded to six patients. Once 2 patients at a dose level experienced DLT, the next lower dose level was the MTD. In the expansion cohort, 13 additional CTCL patients were treated at the MTD.

Toxicity evaluation--Toxicities were determined before treatment and daily for four days and then on days 10, 23, 37, and at follow-up visits by history, physical exams, CBC with differential, serum chemistries. ECG was done before treatment and on day 1 and 4. Blood EBV and CMV PCR titers were obtained before treatment and on day 4, 10, 16, 23, and 37. 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).

Responses were assessed by examinations/photographs/biopsies of the skin, lymph node examinations/biopsies, bone marrow aspirate and biopsy, and CT scans performed before treatment and at one month and every three months and at times of disease progression in the dose escalation phase of the study. In the dose expansion cohort, skin assessments and pictures were obtained pretreatment and

at 1, 3, 6, 9, 12, 18, 24, 36, 48, 60, and 72 months. CTCL response criteria were based on the mSWAT score.[s1] Complete response (CR) required an mSWAT score of 0, normal liver and spleen size, absence of pathologic adenopathy by exam and CT scan, and normal bone marrow biopsy and aspirate. PR must show a 50% reduction in mSWAT and with no new skin lesions and no pathologic involvement of nodes, marrow or visceral organs. Progressive disease (PD) is associated with a 25% increase in mSWAT or new non-skin disease. Stable disease (SD) is absence of CR, PR, or PD.

Pharmacology/immune response/flow cytometry--Resimmune concentrations in serum samples were measured by a bioassay using Jurkat cells.[s2,s3] Cmax, serum half-life and AUC (Area Under the Curve) were determined. The limit of drug detection was 50pg/mL. 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.[s4] This preparation was used as standard for the anti-Resimmune antibody titer assay.[s4] All unknowns, standards, and controls were run in duplicate. Flow cytometry was developed to monitor T cell populations before and after immunotoxin therapy in the clinical trial.[s4]

Statistical analyses--Toxicities are dichotomized as none vs any or none and mild vs moderate to severe. The rates of toxicity, overall response, and CR, as well as their 95% confidence intervals were estimated using an exact binomial method. The mean and standard deviation values of the pharmacokinetic (PK) parameters including maximum concentration (C_{max}) and half-life ($t_{1/2}$) were reported.

Results/Patient characteristics-- Relevant patient demographic, diagnoses and prior treatment information is detailed in Table S1. There were 18 females and 12 males; the median age was 57 years, and the mean age was 58 years, with a range of 20 to 84 years. The patients had received an average of 3 prior therapies including 4 patients with a single prior regimen and two patients with multiple modalities including allogeneic stem cell transplants.

Results/Toxicities-- Adverse events (AEs) attributed to drug treatment at the 2.5 – 11.5μg/kg dose levels as listed in Table S2. 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 vascular leak syndrome or hypoalbuminemia. The VLS worsened over a week and then resolved over several more weeks. Supportive care with albumin infusions and diuretics reduced symptoms. CMV and/or EBV reactivation based on blood PCR assays occurred in seven patients. In six patients, there were no associated symptoms, and the patients responded to gancyclovir orally and/or rituximab intravenously. Six patients experienced isolated elevation of hepatic transminases without hyperbilirubinemia. Four patients 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 on treatment including two patients with hypophosphatemia and one patient each with hypocalcemia and hypomagnesemia. Each patient responded quickly to electrolyte replacement. 14 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 H-1 and H2-histamine antagonists. Symptoms included fever and/or chills. Three patients had transient hypotension, and one patient hypoxemia. All these reactions resolved rapidly after administration of fluids or oxygen, respectively.

There were three Grade 4/5 drug-related toxicities. Two patients with prior history of congestive heart failure redeveloped severe heart failure and died on days 9 and 11. One patient with recent alemtuzumab had EBV reactivation, EBV-induced liver and renal failure, and died on day 29. Patient #10 treated at 5μg/kg developed severe congestive heart failure and vascular leak syndrome after 5 doses and expired on day 11. He had a history of previous congestive heart failure and cardiomegaly. Patient

#18 treated at 11.25μg/kg also developed severe congestive heart failure and vascular leak syndrome after 6 doses and expired on day 9. She had a history of pulmonary hypertension and right ventricular dilatation. Patient #29 was treated at 7.5μg/kg for 8 doses and had EBV reactivation and EBV-induced liver and renal failure and died on day 29. He had a course of alemtuzumab four months before and had 211/μL CD3+CD4+ T cells prior to Resimmune. After these AEs, the protocol was modified to exclude patients with a history of heart disease or recent alemtuzumab. Additional Grade 3 AEs included six patients with EBV and/or CMV reactivation, two patients with hypophosphatemia, and two patients with 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 7.5μg/kg dose was chosen for the expansion cohort. After these AEs, the protocol was modified to exclude patients with a history of heart disease or recent alemtuzumab and no additional deaths or severe AEs were observed. Twenty-six patients received all 8 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 <8 doses during the treatment period were hypotension and hypoalbuminemia with or without hypoxia or congestive heart failure.

Pharmacology-- Serum samples for pharmacokinetic studies were collected on day 1 and 2 for 14 patients and for immune response measurements were done on day 1 for all 30 patients and day 10 - 30 for 18 patients. Blood flow cytometry assays of circulating T cells were done on day 0 and day 4 or 5 for 20 patients. The results of relevant pharmacologic and immunologic and circulating cell populations are shown in Table S3, S4 and S5. Cmax values averaged 7.9 ng/mL with a range from 0 to 41ng/mL, after treatment on day 1. The clearance of Resimmune generally fit a mono-exponential model. Drug clearance was highly variable with $t_{1/2}$ values averaging 39 min with a range of 5 to 66 min. A typical serum concentration disposition curve is shown in Figure S1. Neither Resimmune Cmax nor $t_{1/2}$ values were related to response or toxicity in this small study. Pretreatment concentrations of circulating antibodies was tested 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 (Table S4). In the 27 patients who had Resimmune antibody titers measured after completion of the cycle, antibody titers increased in all except 1 patient. The mean pretreatment antibody titer for the 28 patient treatments with both pre and post antibody levels was 18μg/mL with a range of 0.8 to 251μg/mL, and the mean posttreatment antibody titer was 925μg/mL with a range of 1 to 5451μg/mL. Type or number of prior therapies was not a determinant in the pretreatment antibody titer. Pretreatment antibody titer was weakly inversely related to Cmax and strongly related to T cell depletion with Pearson r= -0.4 for n=14 and Pearson r=0.81 and n=20, respectively, yielding a p=0.16 two-tailed for correlation with Cmax and p<0.0001 two-tailed for correlation with T cell depletion. Neither pretreatment nor post-treatment antibody titer values 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 (Table S5). The percentage T cell compared to baseline is shown and ranged from <0.1% to 69% with a mean of 11%. There was a weak inverse relationship of pretreatment Cmax to T cell depletion with r= -0.4 n=12 and p=0.2 two-tailed. However, there was no correlation of T cell depletion with dose, response or toxicities.

Table S1. Clinical characteristics

Subject	Age (years)/gender	Disease/Stage	Prior therapy
1	47/F	CTCL/IIB	Nitrogen mustard, interferon, bexarotene
2	78/M	CTCL/IB	Cyclophosphamide/doxorubicin/vincristine/prednisone
3	58/F	CTCL/IB	Psoralen/UVA, fludarabine, nitrogen mustard, bexarotene, dexamethasone, gemcitabine, vorinostat
4	48/M	CTCL/IIB	Triamcinolone, bexarotene, nitrogen mustard, UVB, gemcitabine
5	64/F	CTCL/IB	Accutane, bexarotene, UVB
6	73/F	CTCL/IB	Nitrogen mustard, bexarotene
7	39/M	CTCL/IIB	UVB, Clobetasol
8	50/F	CTCL/IV	UVB
9	84/F	CTCL/IV	UVB, interferon, chlorambucil, etoposide, bexarotene
10	69/M	PTCL	Cyclophosphamide/doxorubicin/vincristine/prednisone, prednisone
11	76/M	CTCL/III	Pralatrexate, UVB, corticosteroid cream
12	49/F	PTCL	Radiation, bexarotene, liposomal doxorubicin, romidepsin
13	81/F	CTCL/IV	Gemcitabine, liposomal doxorubicin, bexarotene, vorinostat
14	49/F	CTCL/IV	Nitrogen mustard, bexarotene, photopheresis
15	61/M	CTCL/IB	Nitrogen mustard, interferon, bexarotene, vorinostat, psoralen/UVA
16	51/F	CTCL/IIB	Nitrogen mustard, interferon, bexarotene, gemcitabine, radiation
17	71/M	CTCL/III	Pralatrexate, bexarotene, romidepsin
18	61/F	T-LGL	Cyclosporine
19	20/F	CTCL/IIB	Romidepsin, pralatrexate, gemcitabine
20	52/M	PTCL	Radiation, liposomal doxorubicin, gemcitabine, navelbine, oxaliplatin
21	56/F	CTCL/III	Romidepsin, gemcitabine, brentuximab vedotin
22	60/M	CTCL/IIB	Cyclophosphamide/doxorubicin/vincristine/prednisone, SGN-35, bexarotene, methotrexate
23a,23b	41/F	CTCL/IIB	Bexarotene, methotrexate, nitrogen mustard, interferon, radiation, psoralen/UVA, acetretin
24	70/F	CTCL/IB	Corticosteroid cream, UVB, methotrexate
25	49/F	CTCL/IB	Cyclophosphamide/doxorubicin/vincristine/prednisone, denileukin diftitox, bexarotene, allogeneic transplant
26	75/F	T-PLL	Alemtuzumab
27	54/M	CTCL/IB	Bexarotene, corticosteroid cream
28	75/M	CTCL/III	Photopheresis, interferon, bexarotene, alemtuzumab
29	52/F	CTCL/IB	Cyclophosphamide/doxorubicin/vincristine/dexamethasone /cytarabine/methotrexate, Clobetasol
30	39/M	CTCL/IB	Allogeneic transplant, radiation, fludarabine/melphalan, interferon, photopheresis, bexarotene

Table S2. Dose, dose number and drug-related adverse events

Patient no.	Dose level (μg/kg)	No. doses received	Drug-related adverse events grade 2 or above (CTCAE v4.03 toxicity grade)
1	2.5	8	None
2	2.5	8	Gr 2 chills, Gr 2 fever, Gr 2 AST, Gr 2 ALT, Gr 2 hypocalcemia, Gr 2 hypoalbuminemia
3	2.5	8	Gr 3 opportunistic EBV infection, Gr 2 chills
4	2.5	8	Gr 3 opportunistic EBV/CMV infections, Gr 2 hypoalbuminemia
5	2.5	8	Gr 3 opportunistic EBV/CMV infections, Gr 2 fever, Gr 2 chills, Gr 2 ALT
6	2.5	8	Gr 3 opportunistic EBV infection, Gr 3 SVT, Gr 2 hypoalbuminemia
7	5	8	Gr 3 opportunistic EBV infection, Gr 2 hypoalbuminemia
8	5	8	Gr 3 ALT, Gr 3 AST, Gr 2 hypoalbuminemia, Gr 2 chills, Gr 2 hypotension
9	5	1	Gr 2 chills, Gr 2 hypotension
10	5	5	Gr 5 heart failure, Gr 4 vascular leak syndrome, Gr 3 uremia, Gr 3 hypophosphatemia, Gr 2 fever, Gr 2 hypotension
11	5	8	None
12	5	8	Gr 2 fever
13	5	8	Gr 2 vascular leak syndrome; Gr 2 hypoalbuminemia
14	7.5	8	None
15	7.5	8	Gr 2 fever, Gr 2 chills, Gr 2 AST, Gr 2 hypoalbuminemia
16	7.5	8	Gr 2 hypoalbuminemia
17	11.25	8	Gr 3 opportunistic EBV infection
18	11.25	6	Gr 5 heart failure, Gr 4 vascular leak syndrome, Gr 4 hypoxia, Gr 3 hypoalbuminemia, Gr 3 SVT, Gr 4 hypotension, Gr 3 uremia,
19	7.5	8	Gr 2 fever
20	7.5	8	Gr 2 chills, Gr 2 fever
21	7.5	8	Gr 2 chills, Gr 3 AST, Gr 3 ALT
22	7.5	8	Gr 2 chills, Gr 2 fever
23a	7.5	8	Gr 2 hypoalbuminemia, Gr 2 hypomagnesemia
23b	7.5	8	None
24	7.5	8	Gr 3 hypophosphatemia
25	7.5	8	Gr 3 hypophosphatemia
26	7.5	8	Gr 2 hypotension, Gr 2 hypoxia
27	7.5	3	Gr 2 ALT, Gr 2 vascular leak syndrome
28	7.5	8	Gr 5 opportunistic EBV infection, Gr 4 liver failure, Gr 4 uremia, Gr4 metabolic acidosis
29	7.5	8	Gr 2 chills
30	7.5	8	Gr 2 hypoalbuminemia

Table S3. Resimmune pharmacokinetics*

Patient no	Dose (μg/kg)	Cmax (ng/mL)	Half-life (min)	AUC (ng*min/mL)
1	2.5	2.6	43	194
2	2.5	6.2	52	403
3	2.5	20.9	42	1321
4	2.5	40.7	66	3337
5	2.5	19.1	44	917
6	2.5	30.3	41	2469
7	5.0	---	---	---
8	5.0	0	---	---
9	5.0	1.9	5	28
10	5.0	2.7	44	115
11	5.0	0	---	---
12	5.0	---	---	---
13	5.0	0	---	---
14	7.5	0	---	---
15	7.5	---	---	---
16	7.5	2.1	39	101
17	11.25	0	---	---
18	11.25	15.8	12	126

*AUC, area under the curve. PK samples for patients no. 7, 12 and 15 were not collected.

Table S4. Resimmune anti-diphtheria toxin antibody titers*

Patient no	Pre anti-DT (μg/mL)	Day 30 anti-DT (μg/mL)
1	1.1	108
2	5.2	1015
3	0.9	9
4	1.2	5
5	4.4	330
6	12.7	118
7	4.9	2309
8	61.1	1617
9	3.3	ND
10	4	45
11	45.3	961
12	5.1	651
13	32.2	4059
14	78.7	253
15	14.2	2056
16	3.9	267
17	251.4	492
18	11.3	ND
19	14.7	431
20	22.4	829
21	26.5	1028
22	7.7	ND
23a	2.6	26
23b	1.1	6
24	30.5	5451
25	19.9	2527
26	2.8	47
27	3.1	341
28	0.8	1
29	3.0	2
30	0.8	43

*DT, diphtheria toxin. Follow-up antibody on patients no 10, 11 and 12 on day 10 and patients no 19, 23, 24, 25, 26, 27, 28 and 29 on day 23. Patient no 30 day 30 antibody not done.

Table S5. Resimmune Blood T cell content*

Patient no	Blood T cells day 4/5 relative to day 0 (%)
1	<0.1
2	0.5
3	<0.1
4	<0.1
5	<0.1
6	<0.1
7	<0.1
8	17.5
11	62
12	<0.1
13	<0.1
14	50
15	0.3
16	0.3
17	69
19	1.4
20	4.8
21	5
22	0.4
23a	0.8
23b	0.4
24	10
25	18
26	1
27	1.5
28	0.4
29	<0.1
30	2.2

*Patient no 26, after only 2 doses, treatment discontinued secondary to severe infusion reaction. CD3 positive cells assayed by flow cytometry as in Methods.

Table S6. Best response and follow-up in CTCL*

Subject no	Dose (μg/kg)	Pre-Treatment mSWAT	Overall response	Length of response (mos)
1	2.5	212	PD	---
2	2.5	16	CR	72+
3	2.5	14	CR	72+
4	2.5	150	PD	---
5	2.5	60	PD	---
6	2.5	18	PR	3
7	5.0	14	PR	14
8	5.0	35	CR	60+
9	5.0	90	PD	---
11	5.0	14	PD	---
13	5.0	2	PD	---
14	7.5	26	PD	---
15	7.5	26	CR	38+
16	7.5	109	PD	---
17	11.25	101	PD	---
19	7.5	42	PD	---
21	7.5	100	PD	---
22	7.5	80	PD	---
23a	7.5	43	PR	3
23b	7.5	20	PR	1
24	7.5	25	PR	6+
25	7.5	30	PD	---
27	7.5	35	PR	4+
28	7.5	4	PD	---
29	7.5	35	PD	---
30	7.5	8	PD	---

*Patient #9 had only one dose; patients #10, #12, #20 had T-cell non-Hodgkin's lymphoma and patients #18 and #26 had T-cell leukemias. CR, complete response; PR, partial response; PD, progressive disease.