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

Arthur E. Frankel,[1] Jung H. Woo,[2] Chul Ahn,[1] Francine M. Foss,[3] Madeleine Duvic,[4] Paul H. Neville,[5] and David M. Neville[5]

[1]University of Texas Southwestern Medical Center, Dallas, TX; [2]Baylor Scott & White Health, Temple, TX; [3]Yale University School of Medicine, New Haven, CT; [4]The University of Texas MD Anderson Cancer Center, Houston, TX; and [5]Angimmune, LLC, Bethesda, MD, USA

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Correspondence: arthur.frankel@utsouthwestern.edu

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.