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RESEARCH ARTICLE

Clinical trial of a humanized anti-IL-2/IL-15 receptor β chain in HAM/TSP

Yoshimi Enose-Akahata[1], Unsong Oh[1a], Joan Ohayon[2], Bridgette Jeanne Billioux[1], Raya Massoud[1], Bonita R. Bryant[3], Ashley Vellucci[1], Nyater Ngouth[1], Irene Cortese[2], Thomas A. Waldmann[3,*] & Steven Jacobson[1,*]

[1]Viral Immunology Section, Division of Neuroimmunology and Neurovirology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, 20892
[2]Neuroimmunology Clinic, Division of Neuroimmunology and Neurovirology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, 20892
[3]Lymphoid Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, 20892

Correspondence
Thomas A. Waldmann, Lymphoid Malignancies Branch, Center for CR, NCI, NIH., 10 Center Drive, MSC 1374, Building 10, Room 4N115, Bethesda, MD 20892. Tel: 240-760-6091; Fax: 240-541-4561; E-mail: tawald@helix.nih.gov
Steven Jacobson, Viral Immunology Section, Neuroimmunology Branch, NINDS, NIH., 10 Center Dr, Building 10, Room 5C103, Bethesda, MD 20892. Tel: 301-496-0519; Fax: 301-402-0373; E-mail: jacobsons@ninds.nih.gov

Present address
[a]Department of Neurology, Virginia Commonwealth University School of Medicine, Richmond, Virginia, 23298

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*Contributed equally.

Abstract

Objective: Human T cell lymphotropic virus 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic, progressive, neurological disease. Chronic activation of $CD8^+$ T cells, as evidenced by increased spontaneous lymphoproliferation and HTLV-1-specific cytotoxic T cells, has been demonstrated in HAM/TSP patients. Since IL-2 and IL-15 stimulate memory $CD8^+$ T cell activity, these cytokines have been implicated in the immunopathogenesis of HAM/TSP. In this phase I trial, we evaluated the safety, pharmacokinetics, and ability of Hu-Mikβ1, a humanized monoclonal antibody directed toward the IL-2/IL-15 receptor β-chain (IL-2/IL-15Rβ: CD122), to saturate CD122 and regulate abnormal immune responses in patients with HAM/TSP by inhibition of IL-15 action. **Methods**: Hu-Mikβ1 was administered intravenously at doses of 0.5 mg/kg, 1.0 mg/kg, or 1.5 mg/kg in a total of nine HAM/TSP patients. Five doses of Hu-Mikβ1 were administered at 3-week intervals. The clinical response was evaluated using standardized scales. Viral and immunologic outcome measures were examined including HTLV-1 proviral load, T cell phenotypic analysis and spontaneous lymphoproliferation in HAM/TSP patients. **Results**: There was no significant toxicity associated with Hu-Mikβ1 administration in HAM/TSP patients. Saturation of CD122 by Hu-Mikβ1 was achieved in five out of nine HAM/TSP patients. Administration of Hu-Mikβ1 was associated with inhibition of aberrant $CD8^+$ T cell function including spontaneous lymphoproliferation and degranulation and IFN-γ expression, especially in HAM/TSP patients that achieved CD122 saturation. **Interpretation**: The treatment with Hu-Mikβ1 had a number of immunological effects on HAM/TSP patients although no clinical efficacy was observed. We also did not see any dose-related toxicity.

Introduction

Human T cell lymphotropic virus 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic, progressive, neurological disease.[1,2] Currently, no therapy has been shown to significantly modify the long-term disability associated with HAM/TSP.[3] HTLV-1 proviral DNA load (PVL) is significantly elevated in the peripheral blood and cerebrospinal fluid (CSF) of HAM/TSP patients and is strongly correlated with disease

pathogenesis.[4,5] HAM/TSP is characterized by perivascular inflammatory infiltrates, predominantly $CD8^+$ T cells, in chronic inflammatory lesions of the central nervous system, affecting the spinal cord in particular patients.[6,7] Notably, increased numbers of memory and/or effector $CD8^+$ T cells and HTLV-1 Tax-specific cytotoxic $CD8^+$ T cells were found in the peripheral blood, CSF and spinal cord of HAM/TSP patients.[8–10] Chronic immune activation associated with HTLV-1 infection has been suggested to underlie the pathogenesis of this disorder.

HTLV-1 expresses a transcriptional trans-activator protein, Tax, which induces the expression of a number of the common γ chain family of cytokines and their receptors, such as IL-2/IL-2R and IL-15/IL-15R.[11–13] Both cytokines induce the proliferation and increase the cytolytic activity of NK and $CD8^+$ T cells, and the receptors for IL-2 and IL-15 share the IL-2Rβ and γ chains.[13] IL-2 and IL-15 could also regulate the expression of inhibitory receptors, such as CD244 and Tim-3, leading to regulation of $CD8^+$ T cell differentiation and exhaustion.[14] In particular, IL-15 is critical for the development of NK cells and antigen-specific memory $CD8^+$ T cells and is well characterized for its role in maintaining memory pools of $CD8^+$ T cells.[13] HAM/TSP patients showed high frequency of $CD4^+CD25(IL\text{-}2R\alpha)^+$ T cells which contain high HTLV-1 PVL, express HTLV-1 *tax* mRNA and induce various cytokines.[15,16] The treatment of HAM/TSP patients with anti-Tac, a humanized monoclonal antibody to the IL-2Rα , demonstrated several inhibitory effects on spontaneous lymphoproliferation and HTLV-1 PVL in PBMCs.[17] In addition to IL-2, IL-15 has been suggested to be involved in pathogenesis of HAM/TSP, potentially through upregulation of IL-15.[18–20] Since increased expressions of these critical immune mediators may directly contribute to cell activation and proliferation observed in HAM/TSP patients, IL-15/IL-15R autocrine loop might be a critical therapeutic target for HAM/TSP.

Hu-Mikβ1 is a humanized monoclonal antibody to the β chain shared by the IL-2 and IL-15 receptors (IL-2/IL-15Rβ; CD122). Hu-Mikβ1 was shown to block IL-15 transpresentation and IL-15-induced cell proliferation and was evaluated in a phase I clinical trial in patients with T cell large granular lymphocyte leukemia.[21] Previous *ex vivo* studies demonstrated that Hu-Mikβ1 inhibited abnormal T cell proliferation and HTLV-1-specific cellular immune responses of HAM/TSP patients,[19,20,22] suggesting that Hu-Mikβ1 might contribute to blocking IL-15 action in HAM/TSP patients. Based on its effects, we hypothesized that the administration of Hu-Mikβ1 in HAM/TSP patients would lead to inhibition of abnormal T cell functions. HAM/TSP patients were intravenously treated with Hu-Mikβ1 at doses of 0.5 mg/kg (group 1), 1.0 mg/kg (group 2), or 1.5 mg/kg (group 3) based on the previous clinical trials.[21,23] The primary goals of this phase I trial were to evaluate the safety, pharmacokinetics, and ability to saturate the IL-2/IL-15Rβ and regulate immune responses in HAM/TSP patients following the administration of Hu-Mikβ1.

Methods

Patients and treatment plan

Nine patients with clinically defined HAM/TSP by the WHO criteria [24] were enrolled into the clinical trial of Hu-Mikβ1 in HAM/TSP (NCT00076843). Hu-Mikβ1 was administered intravenously at doses of 0.5 mg/kg (group 1), 1.0 mg/kg (group 2), or 1.5 mg/kg (group 3). Each patient received five doses of Hu-Mikβ1, were administered at 3-week intervals. PBMCs were isolated and cryopreserved in liquid nitrogen until use. CSF was obtained by lumbar puncture and the cells were collected by centrifugation of CSF samples. The study protocol (04-N-0071) was reviewed and approved by the National Institute of Neurological Disorders and the Stroke Institutional Review Board. Prior to study inclusion, written informed consent was obtained from all the participants in accordance with the Declaration of Helsinki. Details on this clinical trial are recorded at clinicaltrials.gov (NCT00076843).

Flow cytometry

For analysis of peripheral blood lymphocyte populations, EDTA-treated whole blood of HAM/TSP patients and healthy volunteers (HVs) were stained with CD3, CD4, CD8, CD14, CD19, CD25, CD27, CD45, CD45RA, CD45RO, CD56, CD95, CD122 (clone Mikβ2 and Mikβ3), CD197 (CCR7), CD244 (all from BD Biosciences), CD279 (PD-1; BioLegend), and Tim-3 (R&D Biosystems). Mikβ1 and Mikβ3 identified noncompeting epitopes on the IL-2/IL-15Rβ, but Mikβ1 and Mikβ2 appeared to recognize the same epitope or very closely related epitopes on the β chain.[21,25] Tax 11-19/HLA-A201 tetramer was provided by National Institute of Allergy and Infectious Diseases Major Histocompatibility Complex Tetramer Core Facility. CMV pp65/HLA-A201 tetramer (Beckman Coulter) was used as control. For detection of phosphorylated STAT5 (pSTAT5), EDTA-treated whole blood were incubated for 15 min at 37°C and lysed with BD Phosflow™ Lyse/Fix buffer (BD Biosciences). After washing and permeabilization with cold 90% methanol on ice for 30 min, the cells were stained with antibodies for CD3, CD4, CD8, and pSTAT5 (all from BD Biosciences). CD107a mobilization assay was performed as previously described.[20] All flow cytometric

analyses were performed using a FACSCalibur or LSR II (both from BD Biosciences). The data were analyzed using FlowJo 10.2 software (FlowJo LLC).

Lymphoproliferation assay

Lymphoproliferation assay was performed as previously described.[26] PBMCs were cultured in triplicate and pulsed after 3 to 5 days of culture with 1 μCi [^{3}H] thymidine. The average cpm from each of the wells was plotted.

HTLV-1 PVL

HTLV-1 PVL was measured using ddPCR (Bio-Rad) as previously described.[27] Primers and probe specific to HTLV-1 *tax* and human ribonuclease P protein subunit 30 were used.

Statistics

The Mann–Whitney test was used to compare: pSTAT5, PD-1, CD244 and Tscm cells between HVs and HAM/TSP patients. Paired t test was used to compare: pSTAT5, PD-1, CD244, and Tscm cells at pretreatment and at post-Hu-Mikβ1 treatment of HAM/TSP patients. All statistical analyses were performed using Prism (GraphPad software).

Results

Patients characteristics

Nine HAM/TSP patients were enrolled into this phase I clinical trial of Hu-Mikβ1. All had a slowly progressive course of neurologic disease. The demographic characteristics of the study population are summarized in Table 1. Mean age of the study population was 52.8 years. The majority of patients were female and were predominantly African American. Mean disease duration of the study population was 11.6 years.

Table 1. Demographics of HAM/TSP patients for HuMikβ1 trial.

Group	Patient No.	Age (year)	Gender	Race/ Ethnicity	Disease duration (year)
1	HAM #1	39	F	Hispanic	2
(0.5 mg/kg)	HAM #2	60	F	Hispanic	23
	HAM #3	48	F	Black/African American	2
	HAM #4	53	F	Black/African American	11
2	HAM #5	47	F	Black/African American	18
(1.0 mg/kg)	HAM #6	62	M	Black/African American	17
	HAM #7	56	M	Black/African American	1
3	HAM #8	70	M	White	27
(1.5 mg/kg)	HAM #9	40	F	Black/African American	3

Safety and clinical response of Hu-Mikβ1

Adverse events in HAM/TSP patients during the trial are summarized in Table 2. Two subjects (dosed at 0.5 mg/kg) developed deep vein thrombosis (DVT) of the lower extremities after the protocol-specified observation period (at three and three and one-half months following the

Table 2. Adverse events occurring in HAM/TSP patients during HuMikβ1 trial.

Group	Adverse event	Grade	Number of events
0.5mg	pruritus	1	1
	flu-like syndrome	1	1
	hypotension	1	1
	hypokalemia	1	1
	tachycardia	1	1
	fever	1	1
	palpitation	1	1
	diarrhea	1	1
	infection	2	1
	decubitus ulcer	2	1
	limb edema	2	1
	substernal pain	2	1
	rash/desquamation[1]	2	1
	thrombosis[1]	3	2
1.0mg	headache, chills, fatigue	1	1
	flu-like symptoms	1	2
	paresthesias	1	1
	herpes labialis	1	1
	prolonged QTCB on EKG	1	1
	rash	1	1
	tooth abscess	2	1
	infection/UTI	2	1
	elevated ALT	2	1
	shoulder pain	3	1
	thrombosis	3	1
1.5 mg	bradycardia	1	1
	low b12	1	1
	elevated RSVP on echo	1	1
	contact dermatitis	1	1
	anemia	1	1
	vaginal spotting	1	1
	lymphopenia	1	1
	lymphopenia	2	1
	elevated troponin	2	1
	elevated troponin	2	1
	post dural puncture headache	4	1

[1]Post study

final administration of Hu-Mikβ1). One subject (dosed at 1.0mg/kg) developed DVT around 2 months following the last administration of Hu-Mikβ1. This was asymptomatic and was diagnosed by Doppler of the lower extremities as required per protocol. While this was reported as possibly related to the research, prolonged immobilization secondary to the underlying myelopathy was identified as a major risk factor for DVT in both subjects and was likely contributory. An independent data safety monitoring board concurred with these observations and recommended prophylactic anticoagulation therapy as well as excluding patients that were nonambulatory.

The clinical responses were evaluated using standardized scales including expanded disability status scale (EDSS), Scripps neurologic rating scale (SNRS), Timed 25-Foot Walk (T25-FW), ambulation index (AI), and Insituto de Pesquisa Clinica Evandro Chagas (IPEC). Clinical effects of Hu-Mikβ1 in the study population are summarized in Table 3. For the all doses, there were no changes in the rate of clinical progression during the study period and no serious infusion toxicities. One patient (HAM#5, dosed at 1.0mg/kg) had a subjective improvement in bladder function (nocturia) at week 3 that was sustained throughout the study.

Table 3. Clinical parameters of HAM/TSP patients at baseline and posttreatment of HuMikβ1.

Group	Patient No.	Time course	EDSS	SNRS	T25-FW	AI	IPEC
1	HAM #1	Baseline	6.5	75	11.2	3	16
		Week 12	6.5	55	27.5	6	18
		Week 18	6	64	16.8	4	18
	HAM #2	Baseline	6	72	10.2	4	13
		Week 12	6	72	8.1	4	13
		Week 18	6	66	8.6	4	13
	HAM #3	Baseline	6.5	68	41.2	6	20
		Week 12	6.5	64	na	6	21
		Week 18	7	65	na	7	22
	HAM #4	Baseline	7	73	na	7	24
		Week 12	7.5	67	na	7	24
		Week 18	8	61	na	8	26
2	HAM #5	Baseline	6.5	47	23.9	6	17
		Week 12	6.5	50	20.6	6	13
		Week 18	6.5	54	18.8	5	13
	HAM #6	Baseline	7	53	na	8	21
		Week 12	7.5	51	na	8	20
		Week 18	7.5	51	na	8	19
	HAM #7	Baseline	8	55	na	8	24
		Week 12	8	51	na	8	23
		Week 18	8	56	na	8	25
3	HAM #8	Baseline	6.5	67	10.4	5	19
		Week 12	6.5	67	9.1[1]	5	19
		Week 18	6.5	67	10.4	5	19
	HAM #9	Baseline	6	69	33.8	5	14
		Week 12	6	69	39.7	5	14
		Week 18	6	69	33.8	5	14

EDSS, Expanded Disability Status Scale; SNRS, Scripps Neurologic Rating Scale; T25-FW, Timed 25-Foot Walk; AI, Ambulation Index; IPEC, Insituto de Pesquisa Clinica Evandro Chagas; na, not applicable.
[1]At 9 weeks

Saturation of CD122 on NK cells of HAM/TSP patients

NK cells express high frequency of CD122. In the nine HAM/TSP patients, CD122 was highly expressed on NK cells (Mikβ2: 81.4–99.1%, Mikβ3: 84.07–99.2%). Mikβ2, shared epitopes with Hu-Mikβ1, showed saturation of IL-2/IL-15Rβ on $CD56^+$ NK cells of a HAM/TSP patient, but Mikβ3 which had noncompeting epitopes with Hu-Mikβ1 did not (Fig. 1A). This result suggested that Hu-Mikβ1 could block IL-2/IL-15Rβ on $CD56^+$ NK cells but did not deplete $CD56^+$ NK cells of a HAM/TSP patient. Group analysis of frequencies of $Mikβ2^+$ $CD56^+$ NK cells in HAM/TSP patients demonstrated that 25% of group 1 (one of four patients), 66.7% of group 2 (two of three patients), and 100% of group 3 patients (two of two patients) showed saturation of CD122 on $CD56^+$ NK cells at week 12 of Hu-Mikβ1 treatment (Fig. 1B). The five patients also showed more than 90% saturation of CD122 on $CD56^+$ NK cells (Fig. 1C). These results demonstrated that Hu-Mikβ1 could saturate IL-2/IL-15Rβ in HAM/TSP patients, which might be in a dose-dependent manner. Consistent saturation of CD122 on $CD56^+$ NK cells were achieved in HAM#5, #6, and #8 during the Hu-Mikβ1 treatment phase of the trial and were maintained up to 3 weeks after the final dose but lost by 6 weeks after the final dose (Fig. 1D, left graph). HAM#1, #4, and #9 demonstrated delayed or partial saturation of CD122 on $CD56^+$ NK cells during the Hu-Mikβ1 treatment period (Fig. 1D, center graph). In the other patients (HAM#2, #3, and #7), any consistent saturation was not observed during the Hu-Mikβ1 treatment phase of the trial (Fig. 1D, right graph). Since the three patients (HAM#2, #3, and #7) demonstrated a transient saturation of CD122 on $CD56^+$ NK cells at week 1 of Hu-Mikβ1 treatment, it was supposed that saturation of CD122 was not maintained up to 3 weeks after Hu-Mikβ1 treatment (data not shown). These results demonstrated that consistent or partial saturation of IL-2/IL-15Rβ on $CD56^+$ NK cells was achieved in most patients during the Hu-Mikβ1 treatment phase of the trial.

Since Hu-Mikβ1 has been shown to block IL-15-induced cell proliferation [21], we asked whether administration of Hu-Mikβ1 might affect frequency of NK cells in HAM/TSP patients. Group analysis showed that the

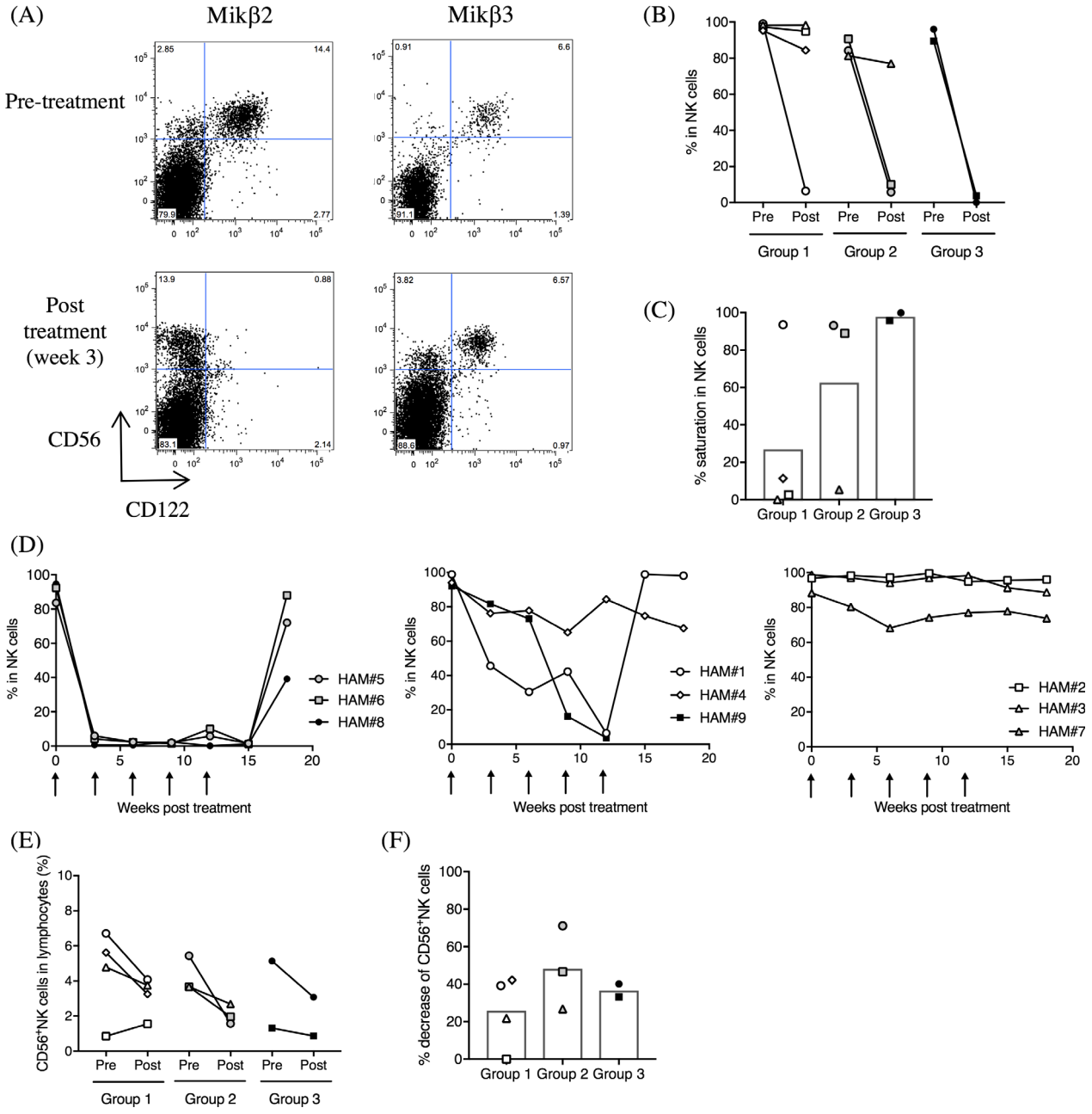

Figure 1. Saturation of CD122 on NK cells. (A) Representative dot plots of CD122 (Mikβ2 and Mikβ3) and CD56 expressions in $CD3^-$ lymphocytes of a HAM/TSP patient. (B) Frequencies of $Mik\beta2^+$ $CD56^+$ NK cells in HAM/TSP patients at pretreatment (pre) and at week 12 of Hu-Mikβ1 treatment (post). (C) Saturation of $Mik\beta2^+$ $CD56^+$ NK cells in HAM/TSP patients (group 1, 2, and 3) at week 12 of Hu-Mikβ1 treatment. Each bar graph represents the mean. (D) Change of frequencies of $Mik\beta2^+$ $CD56^+$ NK cells in HAM/TSP patients (HAM#5, #6 and #8; left graph, HAM#1, #4, and #9; center graph, HAM#2, #3, and #7; right graph) during Hu-MiKβ1 trial. Arrows indicate Hu-Mikβ1 dosing. (E) Frequencies of $CD56^+$ NK cells in HAM/TSP patients at pretreatment (pre) and at week 12 of Hu-Mikβ1 treatment (post). (F) Decrease of $CD56^+$ NK cells in HAM/TSP patients (group 1, 2, and 3) at week 12 of Hu-Mikβ1 treatment. Each bar graph represents the mean

frequencies of $CD56^+$ NK cells were reduced in most HAM/TSP patients after Hu-Mikβ1 administration (Fig. 1E and F). These results indicated that Hu-Mikβ1 administration led to decreased frequency of circulating NK cells.

Saturation of CD122 in $CD8^+$ T cells of HAM/TSP patients

In the nine HAM/TSP patients, CD122 was also detected on $CD8^+$ T cells (Mikβ2: 16.03–55.1%, Mikβ3: 25.0–

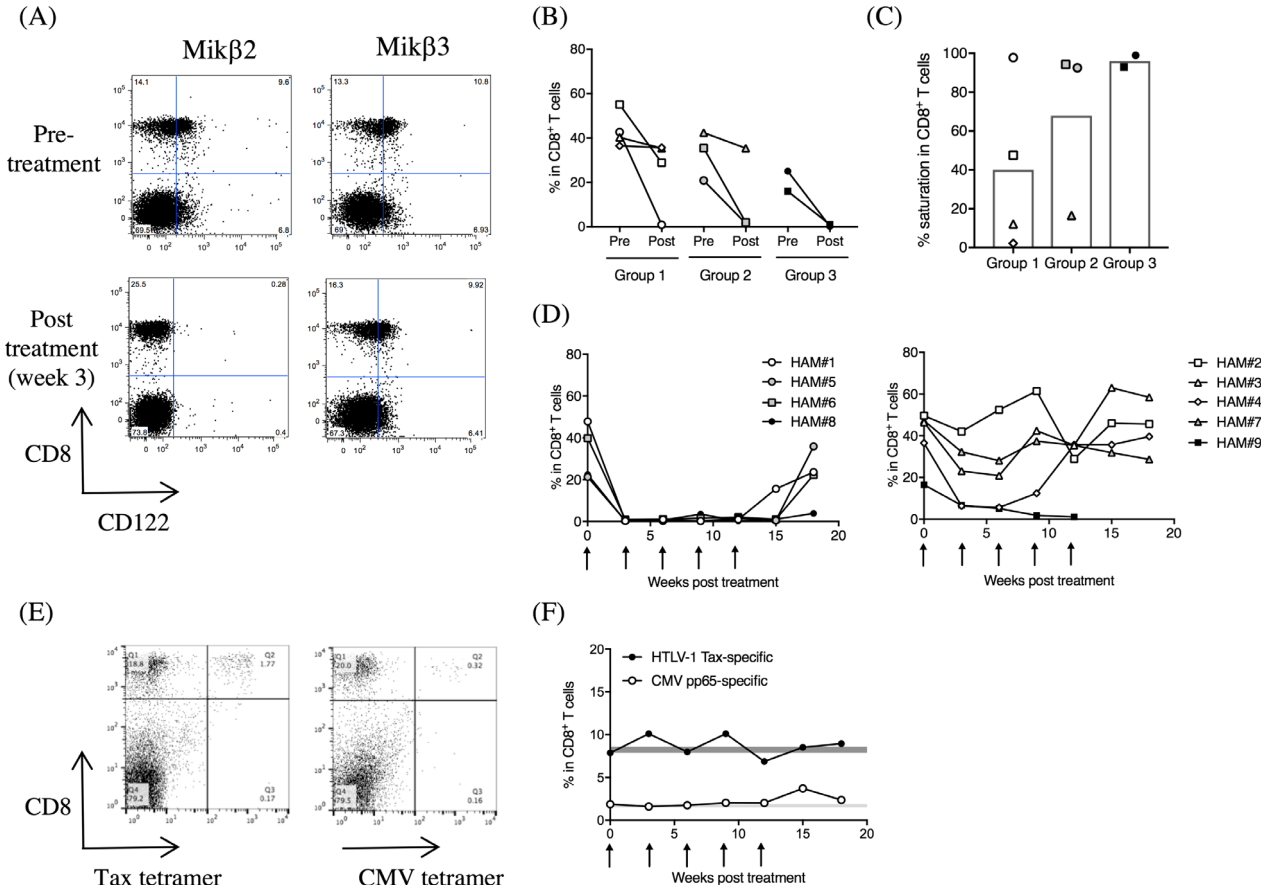

Figure 2. Saturation of CD122 on CD8$^+$ T cells. (A) Representative dot plots of CD122 (Mikβ2 and Mikβ3) and CD8 expressions in CD3$^+$ T lymphocytes of a HAM/TSP patient. (B) Frequencies of Mikβ2$^+$ CD8$^+$ T cells in HAM/TSP patients at pretreatment (pre) and at week 12 of Hu-Mikβ1 treatment (post). (C) Saturation of Mikβ2$^+$ CD8$^+$ T cells in HAM/TSP patients at week 12 of Hu-Mikβ1 treatment. Each bar graph represents the mean. (D) Change of frequencies of Mikβ2$^+$ CD8$^+$ T cells in HAM/TSP patients (HAM#1, #5, #6, and #8; left graph, HAM#2, #3, #4, #7, and #9; right graph) during Hu-Mikβ1 trial. Arrows indicate Hu-Mikβ1 dosing. (E) Tax11-19- and CMV pp65-specific CD8$^+$ T cells in a HAM/TSP patient during Hu-Mikβ1 trial. Representative dot plots of Tax11-19- and CMV pp65-tetramer staining in CD3$^+$ lymphocytes of a HAM/TSP patient. (F) Frequencies of Tax11-19- and CMV pp65-specific CD8$^+$ T cells in a HAM/TSP patient during Hu-Mikβ1 trial. Arrows indicate Hu-Mikβ1 dosing

60.23%). Similar to NK cells, the loss of Mikβ2 binding, not Mikβ3, indicated saturation of CD122 on CD8$^+$ T cells of a HAM/TSP patient following administration of Hu-Mikβ1 (Fig. 2A). Group analysis of frequencies of Mikβ2$^+$ CD8$^+$ T cells in HAM/TSP patients demonstrated that 25% of group 1 (one of four patients), 66.7% of group 2 (two of three patients) and 100% of group 3 patients (two of two patients) showed more than 90% saturation of CD122 on CD8$^+$ T cells at week 12 of Hu-Mikβ1 treatment (Fig. 2B and C). Time course analysis indicated that HAM#1, #5, #6, and #8 sustained the consistent saturation of CD122 on CD8$^+$ T cells during the Hu-Mikβ1 treatment period followed by gradual loss of CD122 saturation on CD8$^+$ T cells during the posttreatment period (Fig. 2D, left graph). In the other patients (HAM#2, #3, #4, #7, and #9), CD122 was partially or transiently saturated on CD8$^+$ T cells (Fig. 2D, right graph). Notably, HAM/TSP patients without significant saturation of CD122 on NK cells also showed a partial saturation of CD122 on CD8$^+$ T cells after Hu-Mikβ1 administration (HAM#2 and #3). These results demonstrated that Hu-Mikβ1 could also saturate IL-2/IL-15Rβ on CD8$^+$ T cells of HAM/TSP patients.

Relatively high frequency of HTLV-1 Tax-specific CD8$^+$ T cells are detected in the peripheral blood of HAM/TSP patients.[10] One HAM/TSP patient (HAM#3, Group 1) expressed the HLA-A201 allele, allowing for the detection of HTLV-1-specific and CMV-specific CD8$^+$ T cells was able to detect in the peripheral blood using Tax11-19/HLA-A201 and CMV pp65/HLA-A201 tetramers, respectively (Fig. 2E). This allowed for reliable monitoring of changes to the frequencies of HTLV-1-

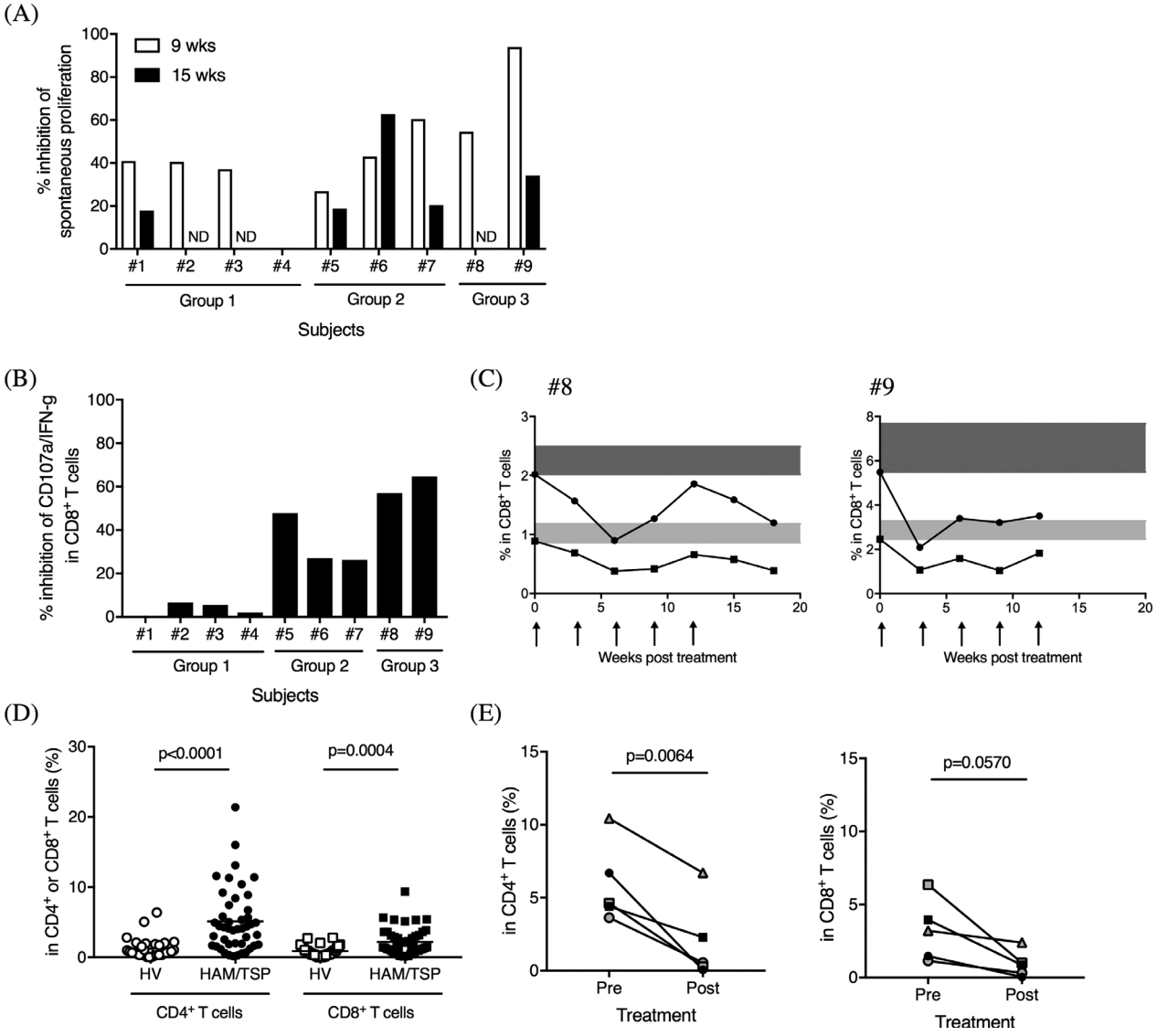

Figure 3. Inhibitory effects of Hu-Mikβ1 on T cell function in HAM/TSP patients. (A) Inhibition of spontaneous lymphoproliferation in HAM/TSP patients at week 9 and week 15 of Hu-Mikβ1 treatment. ND; not done. (B) Inhibition of CD107a and IFN-γ expression in $CD8^+$ T cells of HAM/TSP patients at week 9 of Hu-Mikβ1 treatment. (C) Inhibitory effects of Hu-Mikβ1 on CD107a and IFN-γ expression in $CD8^+$ T cells of HAM/TSP patients (#8 and #9) during Hu-Mikβ1 trial. Arrows indicate Hu-Mikβ1 dosing. The baselines of CD107a expression (closed circles) and both CD107a and IFN-γ expression (closed squires) in PBMC $CD8^+$ T cells of each HAM/TSP patients are highlighted in dark gray and light gray, respectively (D) Comparisons of pSTAT5 in $CD4^+$ and $CD8^+$ T cells of HVs and HAM/TSP patients. The horizontal line represents the mean. (E) Frequencies of pSTAT5 in $CD4^+$ T cells (left) and in $CD8^+$ T cells (right) of HAM/TSP patients (#5-#9) at pretreatment (pre) and at week 9 of Hu-Mikβ1 treatment (post)

and CMV-specific $CD8^+$ T cells during Hu-Mikβ1 treatment. At baseline, 7.88% of $CD8^+$ T cells were HTLV-1 Tax-specific and 1.88% of $CD8^+$ T cells were CMV pp65-specific. The frequencies of both virus-specific $CD8^+$ T cells did not change significantly over the treatment period (Fig. 2F). Of note, the patient (HAM#3) showed only a partial saturation of CD122 on $CD8^+$ T cells (Fig. 2D, right graph).

Inhibitory effects of Hu-Mikβ1 on T cell function of HAM/TSP patients

Spontaneous lymphoproliferation is a well-established measure of *ex vivo* T cell activation for HTLV-1-infected subjects.[28] Partial inhibition of spontaneous lymphoproliferation was detected in the HAM/TSP patients at week 9 and/or week 15 of Hu-Mikβ1 treatment (Fig. 3A). In

particular, patients that achieved full saturation of CD122 during the Hu-Mikβ1 treatment period (e.g., HAM#6, #8, and #9) demonstrated greater than 50% inhibition of spontaneous proliferation at week 9 or week 15 of Hu-Mikβ1 treatment (Fig. 3A).

Spontaneous degranulation and IFN-γ expression has been reported to be increased in $CD8^+$ T cells of HAM/TSP patients and inhibited by Hu-Mikβ1 in *ex vivo* PBMC culture of HAM/TSP patients.[20] HAM/TSP patients in group 3 showed 57.0–64.6% inhibition of spontaneous degranulation and IFN-γ expression in $CD8^+$ T cells at week 9 of Hu-Mikβ1 treatment (Fig. 3B) and varying degrees of inhibition was sustained over the treatment period (Fig. 3C). HAM/TSP patients in the lower dose groups (group 1 and 2) showed less inhibitory effects of Hu-Mikβ1 on spontaneous degranulation and IFN-γ expression in $CD8^+$ T cells (Fig. 3B). These results demonstrated that the administration of Hu-Mikβ1 led to inhibition of $CD8^+$ T cell function in the HAM/TSP patients in a partly dose dependent manner.

It has been reported that PBMCs from HAM/TSP patients showed increased STAT5 activation, as indicated by STAT5 phosphorylation, in short-term (20 h) culture, which was partially inhibited by Hu-Mikβ1.[22] In the current study, we examined pSTAT5 in $CD4^+$ and $CD8^+$ T cells of HVs and HAM/TSP patients using fresh whole blood. As shown in Figure 3D, HAM/TSP patients showed significantly increased pSTAT5 in both $CD4^+$ and $CD8^+$ T cells of peripheral blood compared to HV. When we compared the frequencies of pSTAT5 in $CD4^+$ and $CD8^+$ T cells at pretreatment and at week 9 of Hu-Mikβ1 treatment in the higher dose groups (group 2 and 3), HAM/TSP patients showed significant decreases of pSTAT5 in $CD4^+$ T cells of peripheral blood (Fig. 3E, left) following Hu-Mikβ1 treatment. In $CD8^+$ T cells, pSTAT5 was decreased after Hu-Mikβ1 treatment and approached significance (Fig. 3E, right).

Alternative effects of Hu-Mikβ1 on $CD8^+$ T cell subsets of HAM/TSP patients

Given the inhibitory effects of Hu-Mikβ1 on T cell function, we asked whether Hu-Mikβ1 administration also modulated additional aspects of $CD8^+$ T cell function. In HAM/TSP patients, alternative expressions of various inhibitory receptors, such as PD-1, CD244, and Tim-3, have been demonstrated on $CD8^+$ T cells.[29–32] To determine whether the cells expressing the inhibitory receptors (PD-1, CD244, and Tim-3) were affected by Hu-Mikβ1 administration, we examined the frequencies of $PD\text{-}1^+$, $CD244^+$, and $Tim\text{-}3^+$ cells in $CD8^+$ T cells of HVs and HAM/TSP patients and compared their frequencies in HAM/TSP patients (group 2 and 3) at pretreatment and at week 9 of Hu-Mikβ1 treatment. Compared to HVs, HAM/TSP patients showed higher frequencies of $PD\text{-}1^+$ and $CD244^+$ cells, but not $Tim\text{-}3^+$ cells, in $CD8^+$ T cells (Fig 4A–C). Group analysis of HAM/TSP patients demonstrated significant decrease in $PD\text{-}1^+$ cells in $CD8^+$ T cells at week 9 of Hu-Mikβ1 treatment (Fig 4A). Although there were no significant changes of $CD244^+$ and $Tim\text{-}3^+$ cells in $CD8^+$ T cells of HAM/TSP patients during Hu-Mikβ1 treatment, some HAM/TSP patients showed decrease in $CD244^+CD8^+$ T cells (HAM#5) and $Tim\text{-}3^+$ $CD8^+$ T cells (HAM#6 and 9; Fig 4B and C).

A new subset of human memory $CD8^+$ T cells, stem cell-like memory T cells (Tscm) cells, has been reported to be identified based on expression of CD122 and CD95 in naïve phenotypes and constituted a long-lived, self-renewing lymphocyte population essential for the maintenance of functional immunity, which might be associated with infectious diseases and autoimmune diseases.[33–35] Compared to HVs, the frequency of Tscm was significantly increased in HAM/TSP patients (Fig. 4D). Although there was no significant difference of Tscm frequency in $CD8^+$ T cells of HAM/TSP patients at pretreatment and at week 12 of Hu-Mikβ1 treatment by group analysis, two HAM/TSP patients (HAM#6 and #8) showed decrease in Tscm in $CD8^+$ T cells during Hu-Mikβ1 treatment (Fig. 4D).

HTLV-1 PVL in HAM/TSP patient during Hu-Mikβ1 trial

We also analyzed HTLV-1 PVL in PBMCs of HAM/TSP patients during the trial. HTLV-1 PVL of the HAM/TSP patients was detected at 6.63–43.77% in PBMC before the treatment and the reduction of HTLV-1 PVL at week 12 of Hu-Mikβ1 treatment was 0–44.8% in PBMC of the patients (Fig. 4E, left graph). Of nine HAM/TSP patients, we were able to analyze HTLV-1 PVL in CSF cells of four HAM/TSP patients (HAM#5, #6, #8, and #9) at pretreatment and at week 12 of Hu-Mikβ1 treatment (Fig. 4E, right graph). HTLV-1 PVL was detected at 37.69–72.33% in the CSF cells at pretreatment which was much higher than the PVL in the PBMCs of each patient. At week 12 of Hu-Mikβ1 treatment, HTLV-1 PVL in the CSF cells was trending to decrease (5.2–22.4% reduction) but remained higher than that in the PBMCs of the patients (Fig. 4E, right graph). These results suggested that Hu-Mikβ1 did not directly alter HTLV-1 PVL in HAM/TSP patients.

Discussion

Activation and dysregulation of $CD8^+$ T cells have been implicated in disease progression and pathogenesis of

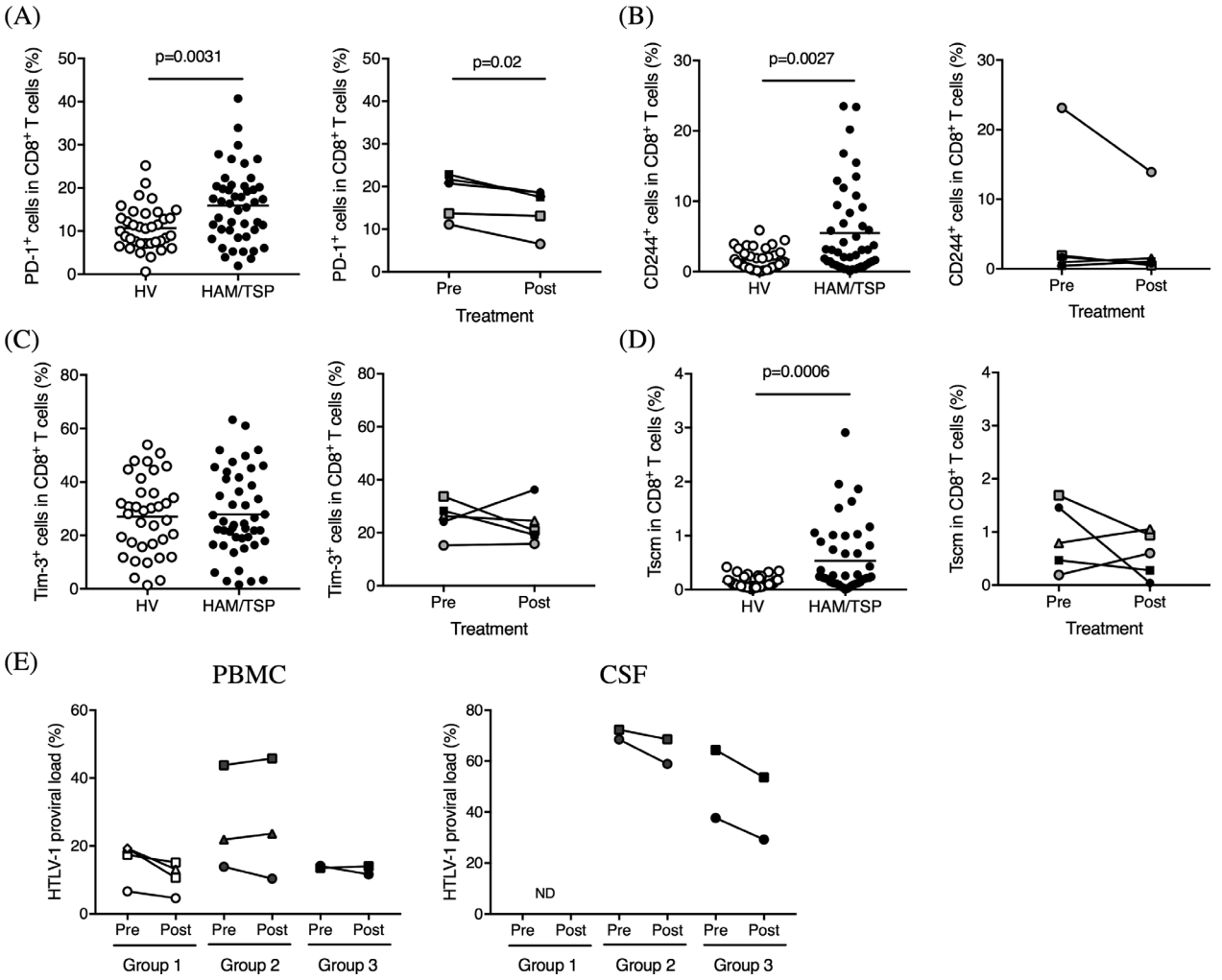

Figure 4. Alternative changes in HAM/TSP patients. (A) Comparison of frequency of PD-1$^+$ cells in CD8$^+$ T cells of HVs and HAM/TSP patients (left graph). The horizontal line represents the mean. Comparison of frequency of PD-1$^+$ cells in CD8$^+$ T cells of HAM/TSP patients at pretreatment (pre) and at week 9 of Hu-Mikβ1 treatment (post) (right graph). (B) Comparison of frequency of CD244$^+$ cells in CD8$^+$ T cells of HVs and HAM/TSP patients (left graph). The horizontal line represents the mean. Comparison of frequency of CD244$^+$ cells in CD8$^+$ T cells of HAM/TSP patients at pretreatment (pre) and at week 9 of Hu-Mikβ1 treatment (post) (right graph). (C) Comparison of frequency of Tim-3$^+$ cells in CD8$^+$ T cells of HVs and HAM/TSP patients (left graph). The horizontal line represents the mean. Comparison of frequency of Tim-3$^+$ cells in CD8$^+$ T cells of HAM/TSP patients at pretreatment (pre) and at week 9 of Hu-Mikβ1 treatment (post) (right graph). (D) Comparison of frequency of Tscm cells in CD8$^+$ T cells of HVs and HAM/TSP patients (left graph). The horizontal line represents the mean. Comparison of frequency of Tscm cells in CD8$^+$ T cells of HAM/TSP patients at pretreatment (pre) and at week 12 of Hu-Mikβ1 treatment (post) (right graph). (E) Comparison of HTLV-1 proviral load in PBMC (left graph) and CSF (right graph) of HAM/TSP patients at pretreatment (pre) and at week 12 of Hu-Mikβ1 treatment (post)

HAM/TSP. In this study, treatment with a humanized monoclonal antibody to the IL-2/IL-15Rβ, Hu-Mikβ1, had several effects on HAM/TSP patients. First, no patients showed any dose-related toxicity and manifested progressive disease during the period of therapy. Two subjects (dosed at 0.5 mg/kg) and one subject (dosed at 1.0mg/kg) developed DVT after the protocol-specified observation period. Although it was not clear whether it was directly related to the treatment, prophylactic anticoagulation therapy was recommended as well as excluding patients that were nonambulatory.

Hu-Mikβ1 administered at 3-week intervals achieved greater than 90% saturation of CD122 on NK cells and CD8$^+$ T cells in five of nine HAM/TSP patients. Saturation of CD122 by Hu-Mikβ1 was more consistently observed among patients receiving the higher doses of Hu-Mikβ1. The results would be consistent with the first phase I clinical trial of Hu-Mikβ1 in patients with T cell

large granular lymphocyte leukemia.[21] Among the five HAM/TSP patients that demonstrated CD122 saturation, three patients demonstrated sustained saturation of CD122 on NK cells and $CD8^+$ T cells during the Hu-Mikβ1 treatment period and then lost saturation of CD122 by 6 weeks after the final dose. Importantly, the higher doses of Hu-Mikβ1 administration provided more inhibitory effects on activated T cell functions of HAM/TSP patients, which are the characteristic features of T cell dysregulation in HAM/TSP patients.[19,20,22,28] In addition, HAM/TSP patients with longer disease duration (such as HAM#5, #6, and #8) seemed to show more effects of Hu-Mikβ1 on CD122 saturation and inhibition of activated $CD8^+$ T cells compared to HAM/TSP patients with shorter disease duration (such as HAM#7), which might be related to chronic activation and/or expansion of $CD8^+$ T cells in the patients. It has been previously reported that Hu-Mikβ1 was able to inhibit the activated T cell functions in *ex vivo* experiments using PBMCs of HAM/TSP patients.[19,20,22] In the current study, our results strongly supported the inhibitory effects of Hu-Mikβ1 on activated T cell functions of HAM/TSP patients *in vivo*, suggesting that Hu-Mikβ1 would effectively inhibit IL-15-driven T cell dysfunction of HAM/TSP patients when IL-2/IL-15Rβ saturation is achieved.

Chronic viral infection has been reported to induce expression of inhibitory molecules that generate negative signals to downregulate the ensuing T cell responses. Expression of multiple distinct inhibitory receptors is associated with greater T cell exhaustion and rapid disease progression.[36] In our cohort, higher frequencies of $PD\text{-}1^+$ cells and $CD244^+$ cells, but not Tim-3, were detected in $CD8^+$ T cells of HAM/TSP patients compared to HVs. During Hu-Mikβ1 treatment, $PD\text{-}1^+$ cells were significantly decreased in $CD8^+$ T cells of HAM/TSP patients. We also demonstrated that higher frequency of Tscm cells was detected in $CD8^+$ T cells of HAM/TSP patients compared to HVs. Tscm cells have similar functions to memory T cells including the ability to proliferate rapidly and release inflammatory cytokines in response to antigen reexposure, and a dependence on IL-15 and IL-7 for homeostatic turnover.[33,34] To achieve long-lived protection against chronic HTLV-1 infection, an adequate number of functionally competent memory $CD8^+$ T cells might be sustained through cytokine-driven homeostatic proliferation. Recently, it has been reported that frequency of $CD8^+$ Tscm cells was increased in patients with acquired aplastic anemia and uveitis, immune-mediated diseases associated with autoreactive cytotoxic $CD8^+$ T cells.[35,37] Intriguingly, Tscm cells in $CD4^+$ T cells have been able to sustain themselves through a process of self-renewal and to reconstitute the identical adult T cell leukemia clones.[38] Although there were no significant changes in the frequency of $CD244^+CD8^+$ T cells and $CD8^+$ Tscm cells after Hu-Mikβ1 administration, it is of interest that Hu-Mikβ1 treatment might be able to modulate $CD8^+$ T cell differentiation and exhaustion in some HAM/TSP patients.

HTLV-1 PVL in the PBMCs did not change as a result of Hu-Mikβ1 treatment whereas HTLV-1 PVL was decreased after treatment with anti-Tac in a previous clinical trial.[17] HTLV-1 infects mainly $CD25^+CCR4^+CD4^+$ T cells and induces functional changes in the infected cells.[15,39,40] A recent report demonstrated that a humanized anti-CCR4 monoclonal antibody decreased the number of HTLV-1-infected cells and the level of inflammatory markers.[41] While both anti-Tac and anti-CCR4 mainly targeted to $CD4^+$ T cells, Hu-Mikβ1 might have less efficiency on $CD4^+$ T cells since the frequency of $CD122^+$ cells in $CD4^+$ T cells was much lower than that in $CD8^+$ T cells. Our results suggest that Hu-Mikβ1 may not directly targeted to HTLV-1 and HTLV-1-infected lymphocytes, but instead modulate T cell dysfunction that characterize HAM/TSP. Combining antiviral therapy with immunotherapies that inhibit T cell dysfunctions might be required to maximize the longevity and effective responses in patients with chronic virus-associated neuroinflammatory disease.

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Author Contribution

YE-A, UO, JO, TAW, SJ design the study and contributed to discussion and paper writing. JO, BJB, RM, UO, IC, TAW coordinated clinical work and patient care. YE-A, AV, NN, and BRB performed the experimental works. YE-A performed statistical analysis. TAW and SJ supervised the project.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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