

Clinical Study Protocol

Phase I/IIa Study of Low Dose Subcutaneous Interleukin-2 (IL-2) for Treatment of Refractory Chronic Graft Versus Host Disease

Takeru Asano^a, Ken-ichi Matsuoka^{a*}, Satoshi Iyama^b, Kazuteru Ohashi^c, Yoshihiro Inamoto^d, Chikako Ohwada^e, Makoto Murata^f, Atsushi Satake^g, Chikamasa Yoshida^h, Koichi Nakaseⁱ, Yasuo Mori^j, and Mitsune Tanimoto^a

^aDepartment of Hematology and Oncology, Okayama University Hospital, Okayama 700-8558, Japan, ^bDepartment of Medical Oncology and Hematology, Sapporo Medical University Hospital, Hokkaido 060-8543, Japan, ^cHematology division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Bunkyo-ku, Tokyo 113-8677, Japan, ^dDepartment of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Chuo-ku, Tokyo 104-0045, Japan, ^eDepartment of Hematology, Chiba University Hospital, Chiba 260-8677, Japan, ^fDepartment of Hematology and Oncology, Nagoya University Graduate School of Medicine, Aichi 466-8560, Japan, ^gFirst Department of Internal Medicine, Kansai Medical University, Osaka 573-1191, Japan, ^hDivision of Hematology, National Hospital Organization Minami-Okayama Medical Center, Okayama 701-0304, Japan, ⁱDivision of Hematology, Ehime Prefectural Central Hospital, Ehime 790-0024, Japan, ^jDepartment of Hematology and Oncology, Kyushu University Hospital, Fukuoka 812-8582, Japan

Chronic graft versus host disease (cGVHD) remains a major problem for long survivors after allogeneic hematopoietic stem cell transplantation (HSCT). Currently, corticosteroid therapy is effective for cGVHD as the first line therapy. However, prolonged therapy with corticosteroids causes various severe adverse events. To develop the new therapeutic strategy of cGVHD, we have launched a multicenter phase I/IIa clinical trial of low dose subcutaneous interleukin-2 (IL-2) for treatment of steroid refractory cGVHD, which is constituted of 2 sequential phases (induction phase and maintenance phase). This study will provide the new therapeutic option for patients with refractory cGVHD after allogeneic HSCT.

Key words: chronic GVHD, allogeneic HSCT, steroid refractory, IL-2

Chronic graft-versus-host disease (cGVHD) is an important late complication after allogeneic hematopoietic stem cell transplantation (HSCT). It is estimated that half of long-term survivors of allogeneic HSCT will develop cGVHD, and it is the cause of death in up to 80% of patients with severe cGVHD [1]. Chronic GVHD is also associated with an impaired quality of life [2]. In addition to donor-recipient disparity of human leukocyte antigen (HLA), the use of unrelated donors (increased minor HLA

antigen disparity), donor-recipient sex disparity and increasing donor age [3], peripheral blood stem cell transplantation (PBSCT) increase the relative risk of cGVHD by 40-50% rather than bone marrow transplantation (BMT) [4]. With the dramatic increase of unrelated PBSCT recently in Japan, cGVHD will remain a major problem affecting long-term survivors after allogeneic HSCT.

Chronic GVHD involve a variety of organs, commonly including skin, liver, eyes, oral mucosa/minor salivary glands, lungs, kidneys, soft tissues and joints,

Received July 7, 2016; accepted August 1, 2016.

*Corresponding author. Phone: +81-86-235-7227; Fax: +81-86-232-8226
E-mail: k-matsu@md.okayama-u.ac.jp (K. Matsuoka)

Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

and show various clinical manifestations. The pathophysiology of cGVHD is not well understood. The increased risk of cGVHD after major or minor HLA mismatched HSCT suggests that the expansion of cytotoxic T cells against these mismatched antigens is a main mechanism of cGVHD. Corticosteroids are the first line therapy for cGVHD. Currently, however, there is no standard second line therapy for cGVHD, and therapy typically requires prolonged administration of corticosteroid combined with other immunosuppressive agents such as calcineurin inhibitors. Despite combined immunosuppressive therapy, only 54% of patients were successfully weaned of corticosteroids at 5 years [5]. Prolonged therapy with corticosteroids often causes various severe adverse events. It is clear that new approach in the therapy of refractory cGVHD is urgently required.

Regulatory T cells (Tregs) are a distinct CD4+ T cell subset of mature T cells, which have an essential role in the control of normal immune tolerance [6,7]. After the development in thymus, mature Tregs constitute 1–5% of the circulating CD4+ T cell population. Tregs can suppress effector CD4+ and CD8+ T cells by various mechanisms (directly; cell contact dependent inhibition of antigen presenting cell function, indirectly; production of inhibitory cytokines such as Interleukin-10 and Transforming growth factor- β) [8]. In murine allogeneic BMT model, infused Tregs suppressed allo-reactive effector T cells and acute GVHD [9,10]. In murine solid tumor model, Tregs did not suppress anti-tumor effect in vivo [11,12]. In humans, patients with cGVHD have relative deficiency of Tregs [13,14]. These results suggest that Tregs can induce selective immune suppression and play an indispensable function of immune tolerance after allogeneic HSCT.

Interleukin-2 (IL-2) is a cytokine critical for the development, expansion and peripheral activity of Tregs [15,16]. The constitutive expression of high-affinity IL-2 receptors enables Tregs to selective respond and activation to low dose IL-2 [17]. The deficiency of Tregs in cGVHD patients was restored by the exogenous administration of low dose IL-2 [18,19]. In these clinical trials, daily low dose IL-2 therapy for 8–12 weeks led to selective Treg expansion and improvement of clinical GVHD symptoms in more than 50% of patients [18,19]. Low dose IL-2 has also impacted on Treg homeostasis (increase of

proliferation, generation from thymus and anti-apoptosis activity) [20]. These results indicate that low dose IL-2 therapy can reversibly correct the abnormal Treg homeostasis as new immune approach after allogeneic HSCT.

Here, in order to develop more safe and durable prolonged low dose IL-2 therapy, we have conducted the new strategy of low dose IL-2 therapy which is composed of 2 sequential phases (induction phase and maintenance phase). In this phase I/IIa study, we will study the safety and efficacy of 2 sequential phases low dose IL-2 therapy in patients with steroid refractory cGVHD.

Treatment Methods

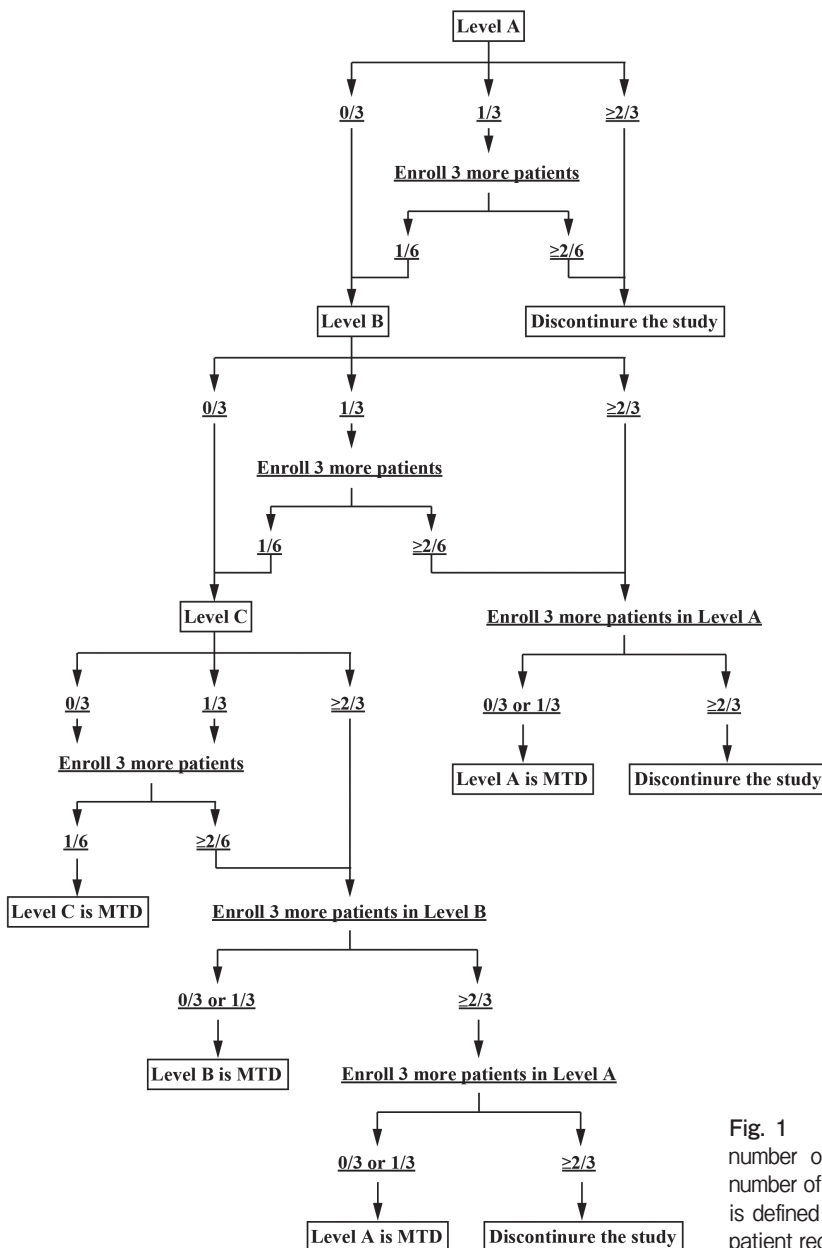
This study is a single arm, non-randomized, open-label phase I/IIa trial of recombinant human IL-2 (UMIN registration number: 000022253). IL-2 (Teceleukin) was supplied by Shionogi, which did not have any input into the manuscript content or the decision to submit the manuscript for publication. This is a multi-center trial involving ten hamatological institutions: Okayama University Hospital, Sapporo Medical University Hospital, Tokyo Metropolitan Cancer and Infectious Diseases Center Komagome Hospital, National Cancer Center Hospital, Chiba University Hospital, Nagoya University Hospital, Kansai Medical University Hospital, Minami-Okayama Medical Center, Ehime Prefectural Central Hospital and Kyushu University Hospital. The co-ordination of the study will be carried out by the Okayama University Hospital, department of Hematology and Oncology. Written informed consent must be obtained by an investigator from the patient before any screening or inclusion procedure. This study was conducted in compliance with the principles of the Declaration of Helsinki, and the protocol was approved by the institutional review boards of each of the participating hospital (The local Ethical Committee Board approval number: 270201).

Treatment is composed of two sequential phases: the induction phase and the maintenance phase. In the induction phase, IL-2 is subcutaneously administrated once per day for 4 weeks. In the sequential maintenance phase, IL-2 is subcutaneously administrated three times per week for following 8 weeks. Patients with safe toxicity profile and clinical benefit could

continue to receive subcutaneous IL-2 for the additional 36 weeks. There are three dose levels of IL-2 in the Phase I part of this study: level A at 3×10^4 , level B at 1×10^5 and level C at 3×10^5 international units/m²/day. Level A is the starting dose, and initially 3 patients are administered. The schema of dose escalation is shown in Fig. 1. The maximum tolerated dose (MTD) of IL-2 is determined in the Phase I part and the MTD is administered in the Phase IIa part of this study. The MTD is defined as the dose

with dose limiting toxicity (DLT) in one or less patient received IL-2. DLT is defined by the following criteria. Severity of toxicity is assessed according to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE v4.0). (1) Grade 4 anaphylaxis: Life threatening anaphylaxis secondary to IL-2, (2) Severe thrombotic microangiopathy (Grade 3: Blood and Marrow Transplant Clinical Trials Network Criteria), (3) Grade 4 non-hematologic toxicity directly related to IL-2 by week 4 of IL-2 treatment, (4) Grade 4

hematologic toxicity, not related to malignant disease relapse, infection or other etiologies (absolute neutrophil count: $ANC < 500/mm^3$ or platelet count $< 25,000/mm^3$), (5) Grade 3 non-hematologic toxicity with intolerance judged by attending physicians, (6) Grade 2 non-hematologic toxicity ≥ 3 days with intolerance judged by attending physicians, (7) Delay to receive IL-2 ≥ 8 days in the induction phase, (8) Progression of cGVHD (Clinical signs of progression of cGVHD while on IL-2, that require the addition of a new immunosuppressive medication or the increase in the corticosteroid dose). The dose determining committee reviews the interim analysis after the dose of each level and then provide decisions regarding the choice of dose to administer to subsequent participants. The dose determining committee will also review all safety data accumulated in the trial at each meeting. Up to 18 participants with refractory cGVHD will be enrolled in the trial to determine the MTD.



Eligibility Criteria

The inclusion and exclusion crite-

Fig. 1 Scheme of dose escalation. #/# indicates the number of patients with dose-limiting toxicity (DLT)/total number of patients. MTD, maximum tolerated dose. The MTD is defined as the dose with DLT in one or less of at least 6 patient received each level of IL-2.

ria are as listed in Table 1.

Endpoints and Statistical Consideration

The primary end point in the phase I part is the detection of the MTD of a 4 week course of IL-2 in patients with cGVHD. The primary end point in the phase IIa part is the proportion of 12-week Failure Free Survival (FFS) in all eligible patients, including the patients in the phase I part, as the index of the efficacy of IL-2. FFS is defined from the date of the first IL-2 administration to the date of the non-relapse death, the relapse of disease and the change of sys-

temic therapy for cGVHD. Kaplan-Meier method will be used to calculate the probability of FFS. The second endpoints are the safety profiles based on CTCAE in 12-week and long-term (≤ 48 weeks) IL-2 administration, the clinical response evaluated by National Institutes of Health consensus criteria and substantial reduction in the steroid dose, and the immune response in terms of increase in Tregs. Safety and tolerability assessments include clinical history, physical examination, body temperature, blood pressure, heart rate, 12-lead electrocardiogram, chest X-ray, clinical laboratory tests, pulmonary function test and adverse event recording. The subset

Table 1 Patient eligibility

Inclusion criteria

- 1) Recipients of allogeneic HSCT with myeloablative or nonmyeloablative conditioning regimens
- 2) Patients must be at least 180 days from the allogeneic HSCT
- 3) Patients with steroid refractory chronic GVHD, defined as persistent symptom of cGVHD despite the use of prednisone at ≥ 1.0 mg/kg/day for at least 2 weeks, ≥ 0.5 mg/kg/day for at least 4 weeks or ≥ 1.0 mg/kg every other day for at least 4 weeks
- 4) Patients with stable dose of corticosteroids for 2 weeks prior to enrollment
- 5) No addition or subtraction of other immunosuppressive medications for 4 weeks prior to enrollment
- 6) Patients with adequate organ function (ANC $> 1,000/\text{mm}^3$, Platelet count $> 50,000/\text{mm}^3$, Absolute lymphocyte count $> 400/\text{mm}^3$, Aspartate aminotransferase $< 2x$ upper limit of normal (ULN), Total bilirubin < 2.0 mg/dl, Serum creatinine $< 2x$ ULN)
- 7) Patient age ≥ 18 years
- 8) Patients with reproductive potential must agree to use an appropriate method of birth control during treatment and for six months after completion of treatment
- 9) Written informed consent

Exclusion criteria

- 1) Ongoing prednisone requirement ≥ 1 mg/kg/day
- 2) Exposure to any immunosuppressive medication in the 2 weeks prior to enrollment (Tumor Necrosis Factor inhibitor, Bortezomib, Rituximab or Imatinib)
- 3) Exposure to any investigational therapy in the 4 weeks prior to enrollment (e.g., extracorporeal photopheresis, ultraviolet or Tamibarotene)
- 4) Post-transplant exposure to any immunomodulatory drug within 180 days prior to enrollment (Antithymocyte globulin, Alemtuzumab, OKT3, Mogamulizumab, Basiliximab, Denileukin difitox, Brentuximab vedotin, Nivolumab, Pembrolizumab, Atezolizumab, Avelumab, Ipilimumab, Abatacept or Natalizumab)
- 5) Active malignant disease relapse
- 6) Active, uncontrolled infection
- 7) Active infection with hepatitis B virus or hepatitis C virus
- 8) Life expectancy < 3 months
- 9) Pregnancy or lactation
- 10) Patients without ability to comply IL-2 treatment regimen
- 11) Uncontrolled cardiac angina or symptomatic congestive heart failure (New York Heart Association: Class III or IV)
- 12) Organ transplant recipient
- 13) Recipients received allogeneic HSCT from HLA-mismatched ($\geq 2/6$) donor, except for umbilical cord blood transplant
- 14) Unstable cardiac angina, cardiac infarction, deep vein thrombosis or cerebral infarction (CTCAE Grade ≥ 3)
- 15) Anticoagulant therapy
- 16) History of severe thrombotic microangiopathy
- 17) Hematological malignancy expressing CD25 (IL-2 receptor)
- 18) History of allergy to biological products (e.g. vaccine)
- 19) Patients judged inappropriate for this study by attending physicians

of lymphocyte (T cells, B cells and NK cells) are measured by flow cytometry following IL-2 treatment to assess the immune response.

Discussion

We have planned this phase I/IIa trial, which will reveal the MTD of low dose IL-2 and whether the strategy of low dose IL-2 therapy which is composed of two distinct phases can maintain tolerance effects in selected patients with steroid refractory cGVHD. We consider it a crucial point in the treatment protocol that intermittent administration of IL-2 after consecutive administration are design to develop more safe and durable prolonged low dose IL-2 therapy. We hope this study will provide novel findings those open the door to a new therapeutic strategy for steroid refractory cGVHD.

Acknowledgments. The authors wish to acknowledge and thank the coordinators and all other investigators who contributed to this study. This protocol writing has been conducted with the support from Center for Innovative Clinical Medicine, Okayama University Hospital. This work was supported by MHLW KAKENHI Grant Number 201433012A.

Reference

- Ratanatharathorn V, Ayash L, Lazarus HM, Fu J and Uberti JP: Chronic graft-versus-host disease: clinical manifestation and therapy. *Bone Marrow Transplant* (2001) 28: 121–129.
- Sutherland HJ, Fyles GM, Adams G, Hao Y, Lipton JH, Minden MD, Meharchand JM, Atkins H, Tejpar I and Messner HA: Quality of life following bone marrow transplantation: a comparison of patient reports with population norms. *Bone Marrow Transplant* (1997) 19: 1129–1136.
- Kollman C, Howe CW, Anasetti C, Antin JH, Davies SM, Filipovich AH, Hegland J, Kamani N, Kernan NA, King R, Ratanatharathorn V, Weisdorf D and Confer DL: Donor characteristics as risk factors in recipients after transplantation of bone marrow from unrelated donors: the effect of donor age. *Blood* (2001) 98: 2043–2051.
- Cutler C, Giri S, Jeyapalan S, Paniagua D, Viswanathan A and Antin JH: Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. *J Clin Oncol* (2001) 19: 3685–3691.
- Koc S, Leisenring W, Flowers ME, Anasetti C, Deeg HJ, Nash RA, Sanders JE, Witherspoon RP, Storb R, Appelbaum FR and Martin PJ: Therapy for chronic graft-versus-host disease: a randomized trial comparing cyclosporine plus prednisone versus prednisone alone. *Blood* (2002) 100: 48–51.
- Fehervari Z and Sakaguchi S: Development and function of CD25+CD4+ regulatory T cells. *Curr Opin Immunol* (2004) 16: 203–208.
- Piccirillo CA and Shevach EM: Naturally-occurring CD4+CD25+ immunoregulatory T cells: central players in the arena of peripheral tolerance. *Semin Immunol* (2004) 16: 81–88.
- Sakaguchi S, Wing K, Onishi Y, Prieto-Martin P and Yamaguchi T: Regulatory T cells: how do they suppress immune responses? *Int Immunol* (2009) 21: 1105–1111.
- Hoffmann P, Ermann J, Edinger M, Fathman CG and Strober S: Donor-type CD4(+)CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. *J Exp Med* (2002) 196: 389–399.
- Powrie F, Carlino J, Leach MW, Mauze S and Coffman RL: A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4+ T cells. *J Exp Med* (1996) 183: 2669–2674.
- Edinger M, Hoffmann P, Ermann J, Drago K, Fathman CG, Strober S and Negrin RS: CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat Med* (2003) 9: 1144–1150.
- Turk MJ, Guevara-Patino JA, Rizzuto GA, Engelhorn ME, Sakaguchi S and Houghton AN: Concomitant tumor immunity to a poorly immunogenic melanoma is prevented by regulatory T cells. *J Exp Med* (2004) 200: 771–782.
- Matsuoka K, Kim HT, McDonough S, Bascug G, Warshauer B, Koreth J, Cutler C, Ho VT, Alyea EP, Antin JH, Soiffer RJ and Ritz J: Altered regulatory T cell homeostasis in patients with CD4+ lymphopenia following allogeneic hematopoietic stem cell transplantation. *J Clin Invest* (2010) 120: 1479–1493.
- Zorn E, Kim HT, Lee SJ, Floyd BH, Liitsa D, Arumugarajah S, Bellucci R, Alyea EP, Antin JH, Soiffer RJ and Ritz J: Reduced frequency of FOXP3+ CD4+CD25+ regulatory T cells in patients with chronic graft-versus-host disease. *Blood* (2005) 106: 2903–2911.
- Malek TR and Bayer AL: Tolerance, not immunity, crucially depends on IL-2. *Nat Rev Immunol* (2004) 4: 665–674.
- Nelson BH: IL-2, regulatory T cells, and tolerance. *J Immunol* (2004) 172: 3983–3988.
- Klatzmann D and Abbas AK: The promise of low-dose interleukin-2 therapy for autoimmune and inflammatory diseases. *Nat Rev Immunol* (2015) 15: 283–294.
- Koreth J, Kim HT, Jones KT, Lange PB, Reynolds CG, Chammas MJ, Dusenbury K, Whangbo J, Nikiforow S, Alyea EP 3rd, Armand P, Cutler CS, Ho VT, Chen YB, Avigan D, Blazar BR, Antin JH, Ritz J and Soiffer RJ: Efficacy, durability, and response predictors of low-dose interleukin-2 therapy for chronic graft vs. host disease. *Blood* (2016) DOI 10.1182/blood-2016-02-702852.
- Koreth J, Matsuoka K, Kim HT, McDonough SM, Bindra B, Alyea EP 3rd, Armand P, Cutler C, Ho VT, Treister NS, Bienfang DC, Prasad S, Tzachanis D, Joyce RM, Avigan DE, Antin JH, Ritz J and Soiffer RJ: Interleukin-2 and regulatory T cells in graft-versus-host disease. *N Engl J Med* (2011) 365: 2055–2066.
- Matsuoka K, Koreth J, Kim HT, Bascug G, McDonough S, Kawano Y, Murase K, Cutler C, Ho VT, Alyea EP, Armand P, Blazar BR, Antin JH, Soiffer RJ and Ritz J: Low-dose interleukin-2 therapy restores regulatory T cell homeostasis in patients with chronic graft-versus-host disease. *Sci Transl Med* (2013) 5: 179ra143.