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Title: A Multicenter Prospective Study of the Efficacy and Safety of Combined Immunosuppressive Therapy with High-Dose Glucocorticoid, Tacrolimus, and Cyclophosphamide in Interstitial Lung Diseases Accompanied by Anti-Melanoma Differentiation-Associated Gene 5-Positive Dermatomyositis

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Conflict of Interest

RN has received research grants from Medical & Biological Laboratories Co., Ltd. MY has received licensing fees from Onkimmune, Ltd. HY has received research grants from Astellas Pharma Inc. YT has received speaker's fees from Boehringer Ingelheim. TH has received research grants from Teijin Pharma Co., Ltd. RU has received consulting fees from Eisai and CAC Croit. KM has received research grants from Eisai and Mitsubishi-Tanabe. MH and MT belong to a department that is financially supported by four pharmaceutical companies (Mitsubishi-Tanabe, Chugai, Ayumi, and UCB Japan). MH has received research grants and/or speakers' fees from Astellas, Brystol-Meyers, Eisai, and Mitsubishi-Tanabe). MT has received scholarship donations from six pharmaceutical companies (Pfizer, Astellas, AbbVie GK, Taisyo Toyama, Takeda, and Eisai). TM has received research grants from Acterion, Astellas, Asahi Kasei Pharma, Ayumi, Chugai, Daiichi Sankyo, Eisai, JB, Mitsubishi-Tanabe, MSD, Nippon Shinyaku, Pfizer, Sanofi, and

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Abstract

Objective: Interstitial lung disease (ILD) accompanied by anti-melanoma differentiation-associated gene 5 (MDA5)-positive dermatomyositis (DM) is often rapidly progressive and associated with poor prognosis. Because there is no established treatment, we prospectively evaluated the efficacy and safety of a combined immunosuppressive regimen for anti-MDA5-positive DM with ILD.

Methods: Adult Japanese patients with newly onset anti-MDA5-positive DM with ILD (n = 29) were enrolled in multiple centers from 2014 to 2017. They were treated with a regimen of high-dose glucocorticoids (GC), tacrolimus, and intravenous cyclophosphamide (IVCY). Plasmapheresis was used if patients worsened after the regimen started. The primary endpoint was six-month survival, compared with that of a historical control (n = 15) of patients with anti-MDA5-positive DM with ILD who received step-up treatment (high-dose GC and stepwise addition of immunosuppressant). Secondary endpoints were 12-month survival rate, adverse events, and changes in laboratory data.

Results: The combined immunosuppressive regimen group showed significantly higher 6-month survival than the step-up treatment group (89% and 33%, respectively, $P < 0.0001$). Over 52 weeks, anti-MDA5 titer, serum ferritin level, percent vital capacity, and chest high-resolution computed tomography scores improved. The combined immunosuppressive regimen group received IVCY nearly 20 days earlier with shorter intervals and tended to receive plasmapheresis more often than patients undergoing step-up treatment (n = 15). Cytomegalovirus reactivation was frequently observed over 52 weeks.

Conclusion: A combined immunosuppressive regimen is effective for anti-MDA5-positive DM with ILD. Plasmapheresis can be used for additional effect in intractable disease. Opportunistic infections should be carefully monitored during treatment.

Introduction

Idiopathic inflammatory myopathies (IIMs) are heterogeneous autoimmune diseases that affect skeletal muscle and various systemic organs, including the skin, lungs, heart, and joints. IIMs have been categorized on the basis of clinical phenotype or histopathological characteristics; however, recent studies have focused on the utility of myositis-specific autoantibodies (MSAs) for subcategorization of IIMs and corresponding clinical management (1). Clinically amyopathic dermatomyositis (CADM) is defined as a manifestation of typical skin lesions of dermatomyositis (DM) with few or no features of myopathy. Anti-melanoma differentiation-associated gene 5 (MDA5) antibody, an MSA, is reportedly strongly associated with CADM (2). Anti-MDA5-positive DM/CADM patients often exhibit rapidly progressive interstitial lung disease (i.e., RP-ILD), which results in substantial mortality due to respiratory failure (3, 4). RP-ILD in patients with anti-MDA5-positive DM/CADM is more frequent in Asian populations than in European or American populations (39%–71% vs. 22%–57%, respectively) (3-10); furthermore, anti-MDA5 is one of the most important prognostic factors in DM/CADM patients with ILD in Asia. The source of RP-ILD has not been fully clarified, but is thought to be affected by genetic susceptibility and

environmental factors (11-14).

A standard treatment for RP-ILD in patients with anti-MDA5-positive DM/CADM has not yet been established. The 6-month survival rate of patients undergoing conventional treatment with glucocorticoids (GC) alone or a combination of GC and additional various immunosuppressants (step-up therapy) is 28%–66% in Asian patients (1, 3, 7). Failure of these treatments seems to be partly related to rapid progression in alveolar damage before achieving sufficient immunosuppression. Recently, some case reports have suggested a combined immunosuppressive therapy might be efficacious; this type of therapy includes high-dose GC, calcineurin inhibitors (CNI), and intravenous cyclophosphamide (IVCY), which are administered beginning in the early phase of RP-ILD with DM/CADM (15-18). In addition, we have observed the effectiveness of such combination therapy in the early stage of RP-ILD in Japanese anti-MDA5-positive DM/CADM patients, compared with those receiving step-up therapy (75% vs. 29%) in our hospital (1). However, there has not been a prospective trial regarding the efficacy and safety of this combination therapy. Thus, we herein conducted a single arm

clinical trial to assess the efficacy and safety of a combined immunosuppressive regimen comprising high-dose GC, tacrolimus, and IVCY for anti-MDA5-positive DM/CADM with ILD. To the best of our knowledge, this is the first prospective trial involving DM/CADM-ILD patients with a particular MSA, anti-MDA5, who show relatively uniform clinical characteristics and pathophysiology.

Patients and Methods

Design overview

This study was conducted at five hospitals between July 1, 2014 and August 31, 2017. Figure 1 outlines the study design. In this clinical study, anti-MDA5-positive DM/CADM patients with ILD who were treated with a combined immunosuppressive regimen (high-dose GC, tacrolimus, and IVCY) were compared with an existing historical control, which comprised patients who were treated with step-up treatment (high-dose GC was initially used and immunosuppressants were added stepwise based on deterioration of the patients' clinical conditions) (control group A). Furthermore, an additional historical control group of patients who were

treated with a combined immunosuppressive regimen without plasmapheresis (control group B), prior to this clinical prospective study, was compared with the group of patients who participated in the prospective study.

DM was diagnosed in accordance with the criteria of Bohan and Peter (19); CADM was diagnosed when patients had typical cutaneous lesions of DM without clinical evidence of myositis and with minimal or no increase in serum creatine kinase (CK), as described previously (20, 21). The diagnosis of ILD was based on respiratory symptoms, physical examinations, chest high-resolution computed tomography (HRCT) findings, and pulmonary function tests. Patients were eligible for the study if they were diagnosed with definite, probable, or possible DM or CADM with ILD—without immunosuppressive treatments—before admission to one of the registered hospitals. Serum anti-MDA5 was detected by immunoprecipitation (IPP) assay using ^{35}S-labeled HeLa cells and confirmed by an anti-MDA5 enzyme-linked immunosorbent assay (ELISA, MESACUP™). Patients with the following conditions were excluded: active tuberculosis, active hepatitis

B virus infection, cancer, or age < 16 years at the time of enrollment. The follow-up period was 52 weeks. This study was conducted in accordance with the Declaration of Helsinki and its amendments, and was approved by the Ethics Committee at each institution and registered in the University Hospital Medical Information Network (UMIN000014344). Written informed consent was obtained from all patients prior to enrollment.

Combined immunosuppressive regimen

The combined immunosuppressive regimen consisted of prednisolone, tacrolimus, and IVCY. The treatment was initiated soon after a patient received a diagnosis of DM or CADM with ILD (Figure 1). Prednisolone was initially administered 1 mg/kg/day for 4 weeks; thereafter, the existing dose was reduced by 10% every 2 weeks when the dose was > 30 mg daily, and every 2–4 weeks when it was < 30 mg daily. Tacrolimus was adjusted to retain 12-hour blood trough levels within the range of 10–12 ng/mL. IVCY was initiated at 500 mg/m^2 of body surface area (BSA) biweekly, then gradually increased to a maximum of 1000 mg/m^2 of BSA; this was implemented with the goal of nadir leukocyte count of 2000–3000/μL or a

50% reduction from baseline. After the sixth administration of IVCY, the interval was extended to 4–8 weeks. The intended number of IVCY administrations was 10–15. Additional therapy was permitted when patients worsened to a condition requiring oxygen administration during treatment; such therapy included plasmapheresis. Plasmapheresis was performed one to three times per week for 3–13 consecutive weeks. During plasmapheresis, 1–1.3 volumes of plasma per session were removed, then replaced with an equivalent amount of fresh frozen plasma (FFP-LR480, Nisseki, Japan) or 5% albumin (Albuminar-5, CSL Behring K. K., Japan). On the day of IVCY, extracellular fluid and sodium 2-mercaptoethane sulfonate (MESNA, Uromitexan, Shionogi & Co., Ltd., Japan) were injected into the patients to prevent hemorrhagic cystitis (22). During treatment, the patients underwent regular monitoring of serum cytomegalovirus (CMV) antigenemia and β-D-glucan. Trimethoprim-sulfamethoxazole (TMP/SMZ) was used for prophylaxis for *Pneumocystis jirovecii* pneumonia (PCP) (23). Patients were treated with antiviral drugs at an early stage if they were positive for CMV antigenemia or showed symptoms due to reactivation of CMV. Patients were treated in the outpatient department after they were in remission and their

prednisolone dosages were < 0.5 mg/kg/day.

Step-up treatment group as a historical control (control group A)

We included a historical control group of anti-MDA5-positive DM/CADM patients with ILD who were treated from August 1, 2001 to December 31, 2008 in Kyoto University Hospital. Anti-MDA5 antibody levels were retrospectively measured using preserved patient sera.

Combined immunosuppressive regimen group without plasmapheresis as an additional historical control (control group B)

We included an additional historical control group of anti-MDA5-positive DM/CADM patients with ILD who received a combined immunosuppressive regimen of high-dose GC, cyclosporine A, and IVCY without plasmapheresis from September 2008 to February 2013 in Kyoto University Hospital.

Outcomes: primary and secondary end points

The primary endpoint was 6-month survival rate, which was compared between the combined immunosuppressive regimen group and the historical

control groups. Secondary endpoints were as follows: 12-month survival rate, adverse events, changes in respiratory functions, and changes in HRCT scores and laboratory data, including serum ferritin level and anti-MDA5 titer, during treatment courses.

Clinical and standard laboratory data

Baseline clinical characteristics, including laboratory data, were recorded at the time of admission. After admission to a hospital, electromyography, as well as muscle and skin biopsies, were performed as soon as possible. Chest HRCT and laboratory tests, including blood analysis and urinalysis, were performed at 0, 4, 8, 12, 16, 24, and 52 weeks after the initiation of treatment; blood gas tests were performed at 0, 12, 24, and 52 weeks, and respiratory function was tested at 0, 12, and 24 weeks. Tacrolimus blood trough levels were measured after 12 hours (C12) by a chemiluminescent enzyme immunoassay (ARCHITECTTMi1000; Abbot, Abbot Park, IL, USA).

Adverse events

Adverse events associated with the study medication were recorded during

the observation periods. Renal disorder during the treatment course was defined as an increase in the level of serum creatinine (s-Cre) to > 1.5-fold greater than that at baseline (24).

Detection of anti-MDA5 antibody in IPP assay

MSAs in sera were determined by IPP assay, as previously described (3). Briefly, 10 μL of patient sera were mixed with 2 mg of protein A Sepharose™ CL-4B (GE Healthcare, Uppsala, Sweden) in 500 mL of IPP buffer [10 mM Tris–HCl at pH 8.0, 500 mM NaCl, 0.1% Nonidet P-40 (NP-40)] and incubated on a rotator device for 2 hours at 4°C. Subsquently, the IgG-coated Sepharose was washed, then suspended in 400 mL of IPP buffer for polypeptide studies. HeLa cells (2×10^7) in 100 mL of methionine-free medium were labelled with 18.5 MBq of [^{35}S] methionine (Perkin Elmer, Waltham, MA, USA) overnight at 37°C; they were then sonicated in 2 mL of IPP buffer and the supernatant was recovered by centrifugation. Antibody-coated Sepharose beads were mixed with 100 mL of [^{35}S] methionine-labelled HeLa cell extracts, then rotated at 4°C for 2 hours. Subsequently, Sepharose beads were washed five times, then suspended in

SDS sample buffer; polypeptides were then fractionated by 6.5% SDS–PAGE and detected by autoradiography.

Anti-MDA5 antibodies in ELISA

Anti-MDA5 titers were measured by anti-MDA5 ELISA (MESACUP™, Medical & Biological Laboratories CO., LTD., Nagoya, Japan), in accordance with the manufacturer's instructions; these measurements were performed at 0, 4, 8, 12, 16, 24, and 52 weeks after the initiation of treatment (2). The cut-off value was 32 U/mL as determined by ROC analysis (25).

Interpretation of chest HRCT images and scores

Chest HRCT scans of the patients were blindly and independently evaluated by trained pulmonologists (KT and TH), in accordance with a previously reported classification (26-28). First, the pulmonologists classified chest HRCT into the following patterns: usual interstitial pneumonia (UIP), probable UIP, non-specific interstitial pneumonia (NSIP), NSIP with organizing pneumonia (OP), OP, or unclassifiable by chest HRCT. Secondly, the pulmonologists scored chest HRCT findings including airspace

consolidation, ground-glass attenuation, and interlobular septal thickening and/or reticular opacity in each of six zones (upper, middle, and lower on both sides) during the treatment courses. Interobserver disagreements were resolved by consensus. Interobserver correlations using Spearman's rank correlation coefficient between the two pulmonologists were 0.79 ($P < 0.0001$) for airspace consolidation, 0.36 ($P = 0.002$) for ground-glass attenuation, and 0.73 ($P < 0.0001$) for interlobular septal thickening and/or reticular opacity.

Statistical Analysis

The Mann–Whitney U test, Student's *t*-test, and Fisher's exact test were performed to assess associations of clinical features in the two groups. Survival was calculated from the date of the initial diagnosis to the date of death by any cause. Cumulative survival rates were estimated by Kaplan–Meier analysis; the log-rank test was also used to compare survival rates. Pearson's correlation coefficient was used to examine the correlation between tacrolimus blood concentration and renal function. A value of $P < 0.05$ was considered to be statistically significant. P value was adjusted by Bonferroni's method for comparison between multiple groups. Statistical

analyses were performed using JMP Pro 12 software (SAS Institute, Cary, NC, USA).

Results

Clinical features of anti-MDA5-positive DM/CADM patients with ILD

We enrolled anti-MDA5-positive DM/CADM patients with ILD who visited any of the five registered hospitals (n = 29) (Figure 1). Two patients dropped out from the treatment regimen because their treatment differed from that of the prescribed regimen during the observation period: administration of IVCY was considerably delayed for one patient, while IVCY was changed to infliximab for the other patient. We established a historical control group who received step-up treatment of immunosuppressants (control group A, n = 15). When the two groups were compared, there were no significant differences in age, sex, serum CK levels, cutaneous symptoms, or muscle symptoms (Table 1). DLco (%) was higher in the combined immunosuppressive regimen group. %VC and FEV_1/FVC did not differ between the two groups. In HRCT findings, OP or unclassifiable patterns were mainly observed in both groups. The HRCT scores did not differ

between the two groups. The levels of serum ferritin, lactate dehydrogenase (LDH), and anti-MDA5 titer were elevated in both groups, relative to standard values.

Next, treatment was compared between the two groups. GC were used for all patients in both groups. However, GC doses were slightly lower in the step-up treatment group. CNI, IVCY, and combinations of these were used less frequently in the step-up treatment group (Supplementary Table 1). IVCY was administered 9.37 ± 1.57 times in the combined regimen group. In IVCY-treated patients, the dose of cyclophosphamide was similar between the two groups (Supplementary Figure 1); however, the IVCY intervals tended to be longer in the step-up treatment group than in the combined regimen group. Furthermore, we compared the timing of treatment initiation in the two groups: the duration was nearly 20 days longer from hospitalization to the beginning of IVCY and CNI in the step-up treatment group, compared with that in the combined immunosuppressive regimen group (Supplementary Table 2, Supplementary Figure 1). The periods from onset to the administration of GC, IVCY, and CNI tended to be longer in the

step-up treatment group than in the regimen group, but this difference was not statistically significant. When the patients worsened, additional treatment was applied.

Plasmapheresis tended to be used for more patients in the combined immunosuppressive regimen group than in the step-up treatment group (31% vs. 7%, P = 0.13), although this difference was not statistically significant (Supplementary Table 1). During the combined immunosuppressive treatment, 11 patients exhibited worsened hypoxemia and required oxygen; of these, nine patients received plasmapheresis (Supplementary Table 1). Plasmapheresis was performed 11.6 ± 3.95 times in total, for 45.6 ± 21.0 days. Among the 11 patients, six patients with plasmapheresis and two patients without plasmapheresis survived for > 6 months.

Primary and secondary outcomes

Significant improvements in 6-month and 12-month survival rates were observed in the combined immunosuppressive regimen group compared to

the step-up treatment group (89% vs. 33%, $P < 0.0001$, and 85% vs. 33%, $P < 0.0001$, respectively) (Figure 2A). All survivors recovered to a state in which oxygenation was not required. Serum levels of C-reactive protein (CRP), LDH, Krebs von den Lungen-6 (KL-6) and ferritin, as well as anti-MDA5 titers, percent vital capacities, and chest HRCT scores gradually improved in the combined immunosuppressive regimen group (Figure 3). All deaths were due to exacerbation of ILD; however, infections, such as CMV reactivation (n = 3), PCP (n = 2), and sepsis (n = 2), seemed to trigger the exacerbation of ILD (Supplementary Table 3). PCP occurred in patients who had stopped prophylactic TMP/SMZ due to its adverse effects. During the ultimate exacerbated state of ILD just before death, three patients (75%) showed very high levels of ferritin. In the step-up treatment group, 10 of 15 patients died. Exacerbation of ILD was the cause of death in all patients. Several infections were observed during the course of treatment in both surviving and deceased patients, but there were no significant differences between groups with respect to the rates of infections (Supplementary Table 4).

Furthermore, the step-up treatment group (control group A) was classified

into three subgroups based on the number of drugs administered within 7 days (0, 1, or 2 of the following: GC, CNI, and IVCY) or 52 weeks (1, 2, or 3 of the following: GC, CNI, and IVCY) after admission to hospitals (Figure 2B, 2C). A higher number of drugs started within 7 days was associated with increased survival. Analysis of subgroups classified based on the number of drugs administered during the 52-week follow-up period showed that the survival of the control group A increased in the following order (from lowest to highest): triple (GC, CNI, and IVCY), mono (GC), and dual (GC and CNI/IVCY) therapy; survival in the prospective regimen group was highest compared to any subgroups of the control group A.

Effectiveness of plasmapheresis

To analyze the effectiveness of plasmapheresis, we compared the prospective regimen group (GC, tacrolimus, and IVCY) allowed plasmapheresis as an additional treatment and the historical control group B treated with the regimen (GC, cyclosporine A, and IVCY) without plasmapheresis. There were no differences in basal clinical features of patients and drug usage between the two groups (Supplementary Tables 5

and 6). The length of the period from admission to the initiation of GC, CNI, and IVCY did not differ between the two groups. However, the length of the period from onset to the initiation of GC, CNI, and IVCY was longer in the prospective regimen group than in the historical control group B (Supplementary Table 7). Six-month and 12-month survival rates tended to be higher in the prospective regimen group than in the historical control group B (89% vs. 71%, $P < 0.09$, and 85% vs. 71%, $P < 0.17$, respectively) (Figure 2D).

Comparison between surviving and deceased patients in the combined immunosuppressive regimen group

When the surviving (n = 23) and deceased patients (n = 4) in the combined immunosuppressive regimen group were retrospectively compared, cutaneous ulcer was more frequently observed in the deceased patients (13% vs. 75%, respectively, $P = 0.02$) (Supplementary Table 8). Respiratory function and the period from disease onset to the initiation of treatment did not differ between the two groups. Serum levels of ferritin (459.9 ± 516.3 ng/mL vs. 2050.3 ± 1772.7 ng/mL, respectively, $P = 0.16$), CRP (0.77 ± 1.2

mg/dL vs. 2.7 ± 1.1 mg/dL, respectively, P = 0.01), CK, and LDH before treatment tended to be higher in the deceased patients. During the treatment course, the levels of ferritin and CRP were persistently higher in the deceased patients (Supplementary Figure 2). Total or airspace consolidation chest HRCT scores showed improvement in surviving patients.

Adverse events during the treatment

No severe adverse events directly caused death among the patients in this study. However, some opportunistic infections, insomnia, renal dysfunction, and electrolyte abnormalities were observed (Tables 2 and 3). While antibiotics were more frequently used in the step-up treatment group, there were no differences with respect to bacterial infection between the combined immunosuppressive regimen group and the step-up treatment group. CMV reactivation was more frequently observed in the combined immunosuppressive regimen group; soon after CMV reactivation was determined, patients were treated with anti-viral drugs until serum CMV antigenemia was ameliorated. There was no difference in the frequency of PCP, even in patients who stopped using TMP/SMZ for prophylaxis. In both

groups, abnormalities in electrolytes were observed. In particular, hyponatremia after IVCY was often observed; some patients experienced muscle pain (data not shown). To prevent reduction of bone density due to long-term administration of GC, activated vitamin D and/or bisphosphonates were typically used. Jaw osteomyelitis or jaw bone necrosis were observed in two patients in the combined immunosuppressive regimen group; both stopped bisphosphonate use. In addition, femoral head necrosis occurred in one patient, which was manifested as reduction of body weight. Hemorrhagic cystitis occurred in four patients in the combined immunosuppressive regimen group. In one patient several viruses were examined, and adenovirus was detected as the cause of cystitis. IVCY was discontinued in all four patients in whom hemorrhagic cystitis occurred; these patients received IVCY 5, 7, 9, and 10 times, respectively. Over 52 weeks of treatment, renal functions were slightly worsened in both groups (Table 3, Supplementary Figure 3), but there was no significant difference between the two groups. Thrombotic microangiopathy (TMA) occurred in two patients; one of these patients experienced end-stage renal failure and began hemodialysis.

We analyzed the influence of the blood concentration of tacrolimus on adverse events such as infection and renal dysfunction in the combined immunosuppressant regimen group. The 12-hour trough level of tacrolimus during the 52-week follow-up period was 7.64 ± 3.45 ng/mL. Mean 12-hour trough levels of tacrolimus tended to be correlated with the rate at which creatinine increased (Pearson's R = 0.49, P = 0.02, Supplementary Figure 4). Therefore, a high blood concentration of tacrolimus might exhibit some influence on renal function. When we analyzed the influence of tacrolimus concentration on adverse effects, classifying the prospective regimen group according to the occurrence of adverse events (Supplementary Table 9), there was no significant difference in 12-hour trough levels of tacrolimus between the two groups, except in patients who showed hyperkalemia.

Discussion

The combined immunosuppressive regimen with high-dose GC, tacrolimus, and IVCY improved survival of anti-MDA5-positive DM/CADM patients with ILD, and was well-tolerated in this trial. The regimen in this study involved

introducing intensive immunosuppressive treatment in the early phase of anti-MDA5-positive DM/CADM; additional supportive therapy, plasmapheresis, was used when respiratory function deteriorated faster than disease control could be achieved. No severe adverse events caused death. However, opportunistic infections, such as CMV reactivation, were frequently observed in patients receiving the combined immunosuppressive regimen; such events often triggered the exacerbation of ILD activity. Indeed, all deceased patients in this study experienced opportunistic infections and subsequent exacerbation of ILD, which led to sustained severe respiratory distress, despite appropriate treatment for infection and evidence that microorganisms had disappeared. Thus, prophylaxis for PCP, careful monitoring for other infections (e.g., by checking CRP, CMV antigenemia, and β-D-glucan) and decisions regarding empirical therapeutic intervention are critical when applying this regimen.

The early use of immunosuppressants, such as cyclosporine A, in combination with GC has been reported to improve survival rates in DM-ILD (29). In addition, combination therapy with IVCY, cyclosporine A, and GC

was shown to be superior to the combination of cyclosporine A and GC (30). In the present study, tacrolimus (in place of cyclosporine A) was included as CNI in the combined immunosuppressive regimen in this trial, a satisfactory effect for anti-MDA5-positive DM/CADM patients with ILD was obtained. In the present study, the administration of immunosuppressants other than GC after hospitalization was nearly 20 days earlier in the combined immunosuppressive regimen group, compared with that in the step-up treatment group. Therefore, treatment delay, even for a few weeks, can greatly affect the outcome in anti-MDA5-positive DM/CADM patients with RP-ILD, suggesting the importance of initiating combination therapy with multiple immunosuppressants as soon as possible in these patients.

Plasmapheresis can be expected as an effective additional treatment (31). Recent studies have shown that many types of inflammatory cytokines and chemokines, such as type 1 interferon (32), interleukin (IL)-6 (33), IL-8, IL-10, IL-15, and IL-18 (32-35), are elevated in the sera of DM/CADM-ILD or anti-MDA5-positive DM-ILD patients (35). In addition, anti-MDA5 titer has been associated with disease activity (15). In this context, we chose

plasmapheresis as an additional supportive therapy with the aim of removing the elevated cytokines/ chemokines, as well as anti-MDA5 antibody, the latter of which might also contribute to the onset or exacerbation of disease. Previously, a randomized controlled trial showed no effect of plasmapheresis on polymyositis or DM, but the enrolled patients were heterogeneous and its endpoints were measurements of muscle disease activity (36). Notably, recent case reports have suggested the efficacy of plasmapheresis in patients with anti-MDA5-positive RP-ILD (37-39). Polymyxin B-immobilized fiber column direct hemoperfusion (PMX-DHP) has also been suggested as effective for patients with ILD in CADM or anti-MDA5-positive RP-ILD (40, 41), although the effect was controversial (42, 43). In the future, prospective studies comparing between patients with and without plasmapheresis are necessary.

The adverse effect of renal dysfunction showed gradual and irreversible onset (Table 3, Supplementary Figure 3). It might be a result of drug treatment, especially that of CNI known to cause renal dysfunction, or intensive administration of cyclophosphamide. TMA occurred in two patients,

one of whom showed progression of renal dysfunction despite discontinuation of tacrolimus; in that patient, TMA resulted in end-stage renal failure. TMA can be caused both by tacrolimus and by severe DM disease activity. When renal function seems to deteriorate seriously, it may be necessary to reduce or withdraw the potentially contributing medication. In the future, a more detailed investigation of this regimen is needed to avoid overtreatment in patients whose disease activity can be well-controlled without such intensive immunosuppression.

There were several limitations in our study. First, this was a historical controlled study, because the enrollment of concurrent controls was not possible, based on ethical limitations. Second, plasmapheresis was selected at the attending physician's discretion, so there may have been considerable variation in its application. Third, there was a small number of patients enrolled in this trial, and all were of Japanese ethnicity. Because genetic and environmental backgrounds may contribute to the frequency and severity of ILD in anti-MDA5-positive patients (11-14), it is necessary to investigate therapeutic adaptations and potential effects in larger populations with

different ethnicities. Moreover, further prospective studies are needed, with the combination of immunosuppressants arranged in accordance with the classification of patients' prognostic factors because the triple regimen will be overtreatment for a part of anti-MDA5-positive patients.

In summary, we showed the efficacy and safety of a combined immunosuppressive regimen that included high-dose GC, tacrolimus, and IVCY in Japanese anti-MDA5-positive DM/CADM patients with RP-ILD. The early detection of anti-MDA5, administration of concomitant immunosuppressants, and monitoring of infection are important. Further investigations are needed to aid in detailed adaptation of the regimen, as well as to determine the efficacy of plasmapheresis as an additional therapy.

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Author contribution statement

TM and RN conceived the study design. HT and RN analyzed the data. HT and RN wrote the main manuscript text. HT, RN, YH, YI, MY, HY, SH, TN, ES, KH, YT, MK, SA, KM, MH, MT, KO, and TM contributed to collection of samples and/or data. KT and TH scored chest HRCT. RU contributed to statistical analysis. SI measured ELISA of anti-MDA5. All authors reviewed the manuscript.

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Figure legends

Figure 1.

A) Flow diagram of participants.

B) Combined immunosuppressive therapy regimen for anti-MDA5-positive DM/CADM with RP-ILD.

High-dose prednisolone (PSL), oral tacrolimus (TAC), and intravenous

cyclophosphamide (IVCY) are used in combinations.

Figure 2. Overall survival in anti-MDA5-positive DM/CADM patients.

Cumulative survival rates were analyzed by the Kaplan–Meier test.

A: A prospective regimen group (the combined immunosuppressive regimen group, n = 29, solid line) and a control group A (the step-up treatment group, n = 15, dotted line).

B: Survival rates stratified by number of drugs administered within 7 days after hospital admission. Line graphs show a prospective regimen group (the combined immunosuppressive regimen group, n = 29, solid line) and a control group A (the step-up treatment group): dashed line, no drug (n = 3); dashed dotted line, 1 drug (n = 8); dotted line, 2 drugs (n = 4).

C: Survival rates stratified by number of drugs administered within 52 weeks after hospital admission. Line graphs show a prospective regimen group (the combined immunosuppressive regimen group, n = 29, solid line) and a control group A (the step-up treatment group): dashed line, 1 drug (n = 4); dashed dotted line, 2 drugs (n = 5); dotted line, 3 drugs (n = 6).

D: Comparison of survival rates on the additional effect of plasmapheresis Line graphs show a prospective regimen group (the combined immunosuppressive regimen group with/without plasmapheresis, n = 29, solid line), a control group A (the step-up treatment group, n = 15, dotted line), and a control group B (the combined immunosuppressive regimen group without plasmapheresis, n = 17, dashed dotted line).

*indicates $P < 0.0001$, + indicates censored patients

Figure 3. Laboratory data, respiratory function, and chest high-resolution computed tomography scores in the combined immunosuppressive regimen group

Box-plot diagrams show median ± quartile of laboratory data and respiratory function (A), and chest high-resolution computed tomography (HRCT) scores (B) during the treatment courses. The data were compared between different time points by using paired Student's *t*-test, where $P < 0.0125$ (*, A) or < 0.0167 (**, B) were considered to be significantly different

when adjusted using Bonferroni's method. Dotted lines show cut-off values. CRP, C-reactive protein; LDH, lactate dehydrogenase; KL-6, Krebs von den Lungen-6; %VC, percent vital capacity

Table 1 Clinical features of patients at the time of enrollment

	Combined immunosuppressive regimen (n = 29), n, %	Step-up treatment (n = 15), n, %	P value
Diagnosis	DM: 14 (48%) CADM: 15 (52%)	DM: 9 (60%) CADM: 6 (40%)	0.54
Onset type*	Acute: 8 (30%) Subacute: 15 (56%) Chronic: 4 (15%)	Acute: 3 (20%) Subacute: 7 (47%) Chronic: 5 (33%)	--
Female sex	19 (66%)	12 (80%)	1
Age, years	53.4 ± 13.5	57.6 ± 9.08	0.40
BMI	21.1 ± 3.06	21.3 ± 3.18	0.79
Heliotrope rash	13 (46%)	7 (54%)	0.74
Gottron's sign	28 (97%)	13 (87%)	0.26
Skin ulcer	7 (24%)	4 (57%)	0.17
Proximal muscle weakness	13 (46%)	8 (53%)	0.75
CK (U/L)	284.7 ± 324.6	357.3 ± 484.3	0.76
Ferritin (ng/mL)	689.7 ± 973.9	1428.4 ± 1895.7	0.26
LDH (U/L)	390.6 ± 138.9	410 ± 145.2	0.85
CRE (mg/dL)	0.58 ± 0.11	0.6 ± 0.2	0.44
CRP (mg/dL)	1.47 ± 2.04	1.5 ± 1.6	0.49
ESR (mm/hour)	44.8 ± 22.9	51.9 ± 27.8	0.42
KL-6 (U/mL)	689.7 ± 973.9	951.7 ± 818.5	0.79
SP-D (ng/mL)	64.6 ± 57.2	54.8 ± 40.5	0.98
PaO_2 (mmHg)	80.3 ± 13.9	76.0 ± 7.9	0.45
Anti-MDA5 (U/mL)	205.0 ± 46.3	171.9 ± 31.3	0.03
%VC (%)	78.8 ± 16.1	84.2 ± 10.8	0.29
FEV_1/FVC (%)	82.6 ± 8.40	89.6 ± 14.7	0.36
DLco (mL/min/mmHg)	16.0 ± 10.4	11.1 ± 2.9	0.31
DLco (%)	62.0 ± 14.9	44.6 ± 13.7	0.045
HRCT	N = 28	N = 9	
HRCT patterns	NSIP + OP: 3 (11%) OP: 11 (39%) Unclassifiable: 14 (50%)	NSIP + OP: 1 (11%) OP: 5 (56%) Unclassifiable: 3 (33%)	0.75
Total HRCT score	10.8 ± 6.64	12.4 ± 9.69	0.92
Airspace consolidation	3.87 ± 3.63	3.24 ± 2.49	0.75
Ground-glass attenuation	4.67 ± 2.97	6.99 ± 6.59	0.71
Interlobular septal thickening and/or reticular opacity	2.25 ± 3.08	2.18 ± 3.18	0.34

DM, dermatomyositis; CADM, clinically amyopathic dermatomyositis; CK, creatinine kinase; LDH,

lactate dehydrogenase; CRE, creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; KL-6, Krebs von den Lungen-6; SP-D, pulmonary surfactant protein D; PaO_2, partial pressure of arterial oxygen; MDA5, melanoma differentiation-associated gene 5; %VC, percent vital capacity; FEV_1/FVC, forced expiratory volume in 1 second; DLco, diffusing capacity for carbon monoxide; HRCT, high-resolution computed tomography; OP, organizing pneumonia; NSIP, non-specific interstitial pneumonia.

*Onset type was classified into acute (within 1 month), subacute (1 to 3 months), and chronic (over 3 months). Data were expressed as mean ± standard deviation for variables. Analysis was performed by either Mann–Whitney U test for continuous variables or Fisher's exact test for nominal variables.

Table 2. Adverse events during immunosuppressive treatment

	Combined immunosuppressive regimen (n = 27), n, %	Step-up treatment (n = 15) n, %	P value
Infection (total)	23 (85%)	12 (80%)	0.69
Bacterial infection	10 (37%)	5 (33%)	1
CMV	23 (85%)	5 (33%)	0.0015
HSV/VZV	2 (7%)	2 (13%)	0.61
Candidiasis	15 (56%)	5 (33%)	0.21
Aspergillus	2 (7%)	0 (0%)	0.53
PCP	3 (11%)	2 (13%)	1
Other fungal infections	1 (4%)	2 (13%)	0.29
Usage of antibiotics	13 (48%)	12 (80%)	0.056
Prophylaxis of PCP by TMP/SMZ	24 (89%)	12 (80%)	0.65
Diabetes mellitus	19 (70%)	8 (53%)	0.33
Hyperglycemia	20 (74%)	14 (93%)	0.22
Hyperlipidemia	24 (89%)	13 (87%)	1
Insomnia	22 (81%)	10 (67%)	0.45
Compression fracture	1 (4%)	0 (0%)	1
Femoral head necrosis	1 (4%)	0 (0%)	1
Hypertension	9 (33%)	5 (33%)	1
TMA	2 (7%)	0 (0%)	0.53
Hemorrhagic cystitis	4 (15%)	0 (0%)	0.28
Electrolyte abnormality	24 (89%)	13 (87%)	1
Hyponatremia	22 (81%)	12 (80%)	1
Hypokalemia	14 (52%)	4 (27%)	0.193
Hyperkalemia	12 (44%)	9 (60%)	0.52
Jaw osteomyelitis/jaw bone necrosis	2 (8%)	0 (0%)	0.52

CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus; PCP, pneumocystis pneumonia; TMP/SMZ, Trimethoprim-sulfamethoxazole; TMA, thrombotic microangiopathy.

Analysis was performed by either Mann–Whitney U test for continuous variables or Fisher's exact test for nominal variables.

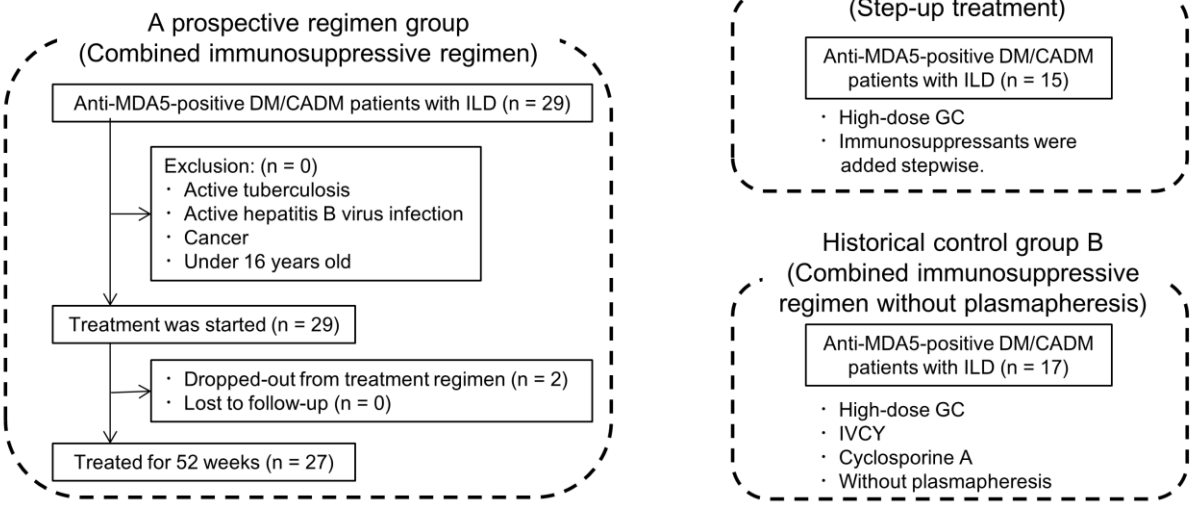
Table 3.Alteration of estimated glomerular filtration rate during treatment courses

	eGFR (0 w)	eGFR (52 w)	P value
Combined immunosuppressive regimen (n = 29)	98.1 ± 23.9	75.3 ± 15.7	5.7×10^{-6}
Step-up treatment (n = 15)	97.4 ± 26.5	67.7 ± 20.2	0.025

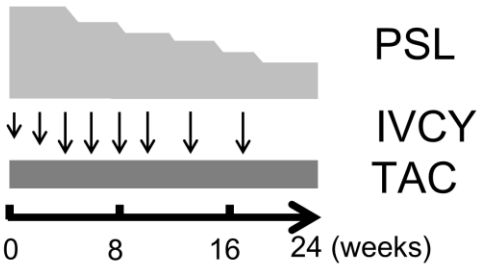
eGFR: estimated glomerular filtration rate (mL/min/1.73 m^2)

Analysis was performed by paired Student's *t*-test.

A) Flow diagram of participants



B) Combined immunosuppressive regimen

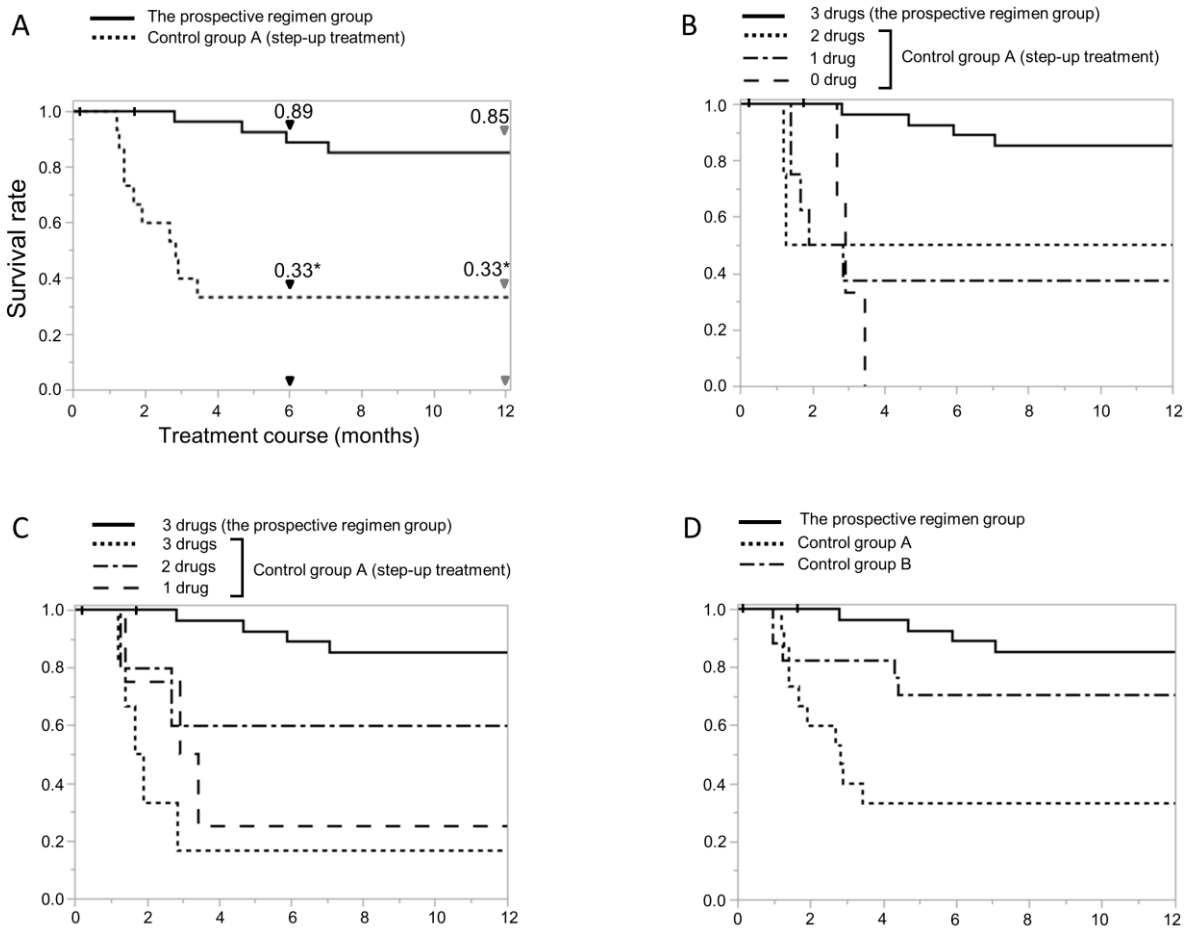


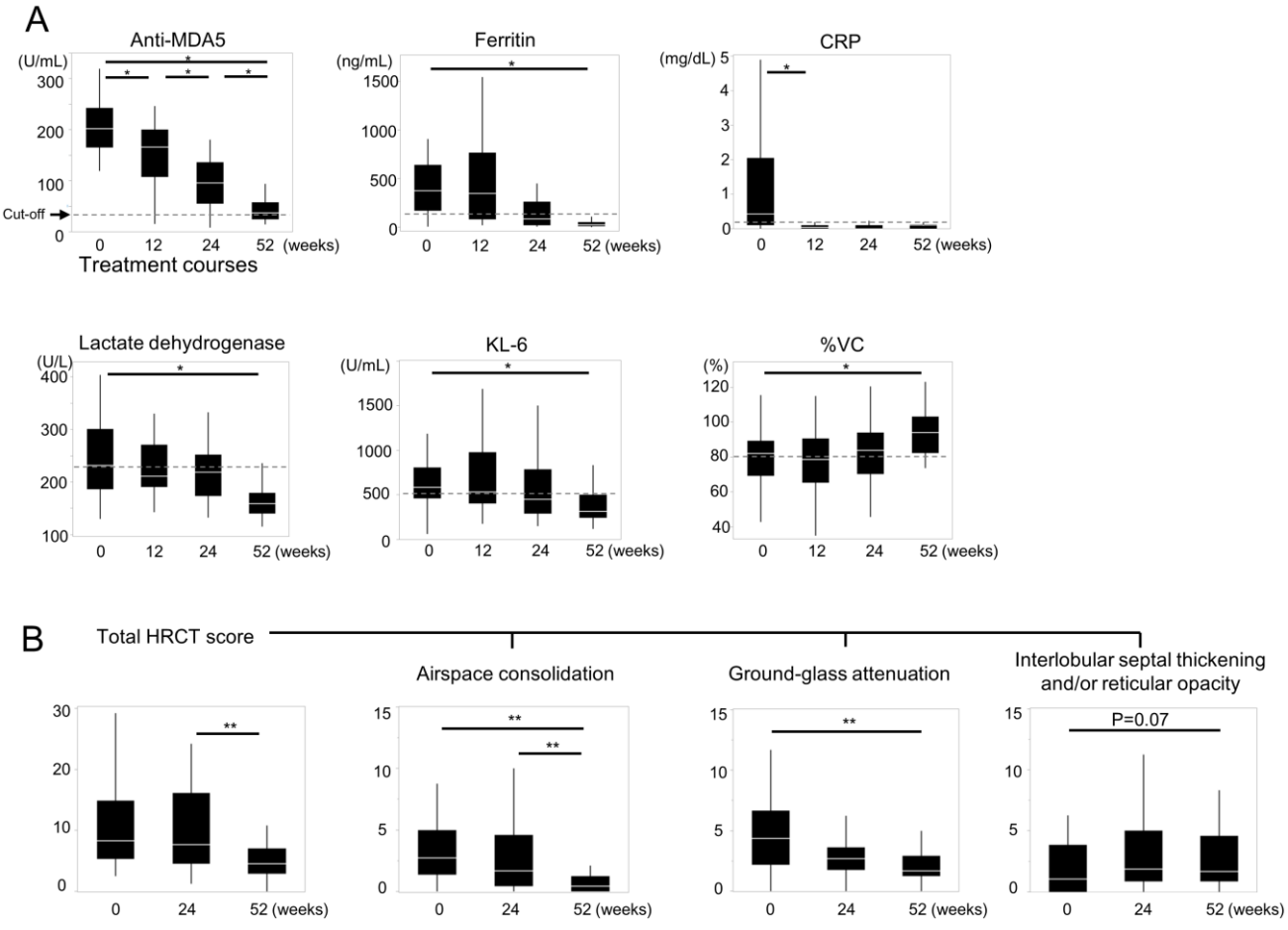
Prednisolone:
1 mg/kg/day (4 weeks), gradually reduced

Intravenous cyclophosphamide:
500–1000 mg/m^2 per 2 weeks, six times
Every 4–8 weeks to a total of 10–15 times

Tacrolimus:10–12 ng/mL (12-h trough)

Additional therapy is allowed.


A
The prospective regimen group
Control group A (step-up treatment)
Survival rate
0.89
0.85
0.33*
0.33*
Treatment course (months)
B
3 drugs (the prospective regimen group)
2 drugs
1 drug
0 drug
Control group A (step-up treatment)
C
3 drugs (the prospective regimen group)
3 drugs
2 drugs
1 drug
Control group A (step-up treatment)
D
The prospective regimen group
Control group A
Control group B


A
Anti-MDA5
(U/mL)
300
200
100
Cut-off
0
0 12 24 52 (weeks)
Treatment courses
Ferritin
(ng/mL)
1500
1000
500
0
0 12 24 52 (weeks)
CRP
(mg/dL) 5
4
3
2
1
0
0 12 24 52 (weeks)
Lactate dehydrogenase
(U/L)
400
300
200
100
0 12 24 52 (weeks)
KL-6
(U/mL)
1500
1000
500
0
0 12 24 52 (weeks)
%VC
(%)
120
100
80
60
40
0 12 24 52 (weeks)
B
Total HRCT score
Airspace consolidation
Ground-glass attenuation
Interlobular septal thickening and/or reticular opacity
30
20
10
0
0 24 52 (weeks)
15
10
5
0
0 24 52 (weeks)
P=0.07

Supplementary Table 1

Comparison of treatment between the combined immunosuppressive regimen and the step-up treatment groups

Treatment	Prospective regimen group: Combined immunosuppressive therapy (n = 29), n, %	Control group A: Step-up treatment (n = 15), n, %	P value
PSL	29 (100%)	15 (100%)	--
PSL (mg/day)	56.5 ± 10.3	41.7 ± 19.5	0.04
IVCY	29 (100%)	8 (53%)	0.0002
Calcineurin inhibitors	29 (100%)	9 (60%)	0.0007
IVCY + Calcineurin inhibitors	29 (100%)	6 (40%)	<0.0001
Additional treatment			
Plasmapheresis	9 (31%)	1 (7%)	0.13
Polymyxin B absorption	0 (0%)	1 (7%)	0.36
IVIG	0 (0%)	2 (14%)	0.12
Infliximab	1 (3%)	0 (0%)	1

PSL, prednisolone; IVCY, intravenous cyclophosphamide; IVIG, intravenous immunoglobulin.
Analysis was performed by either Mann–Whitney U test for continuous variables or Fisher's exact test for nominal variables.

Supplementary Table 2

Periods of drug initiation in patients treated with immunosuppressant

Periods (days) From	Drugs to start	Prospective regimen group: Combined immunosuppressive regimen (n = 29)	Control group A: Step-up treatment (n = 15)	P value
Admission to hospital	GC	3.3 ± 5.3	12.5 ± 28.4 (n = 15)	0.04
	IVCY	5.7 ± 5.5	29.1 ± 18.2 (n = 8)	6.0×10^{-4}
	Calcineurin inhibitors	3.8 ± 5.5	20.6 ± 16.7 (n = 9)	0.047
Onset	GC	93.4 ± 50.1	136.1 ± 134.4 (n = 15)	0.63
	IVCY	100.7 ± 47.2	115.8 ± 55.4 (n = 8)	0.67
	Calcineurin inhibitors	98.0 ± 47.5	171.0 ± 196.9 (n = 9)	0.96

GC, glucocorticoid; IVCY, intravenous cyclophosphamide.

Analysis was performed by Mann–Whitney U test.

Supplementary Table 3.

Deceased patients in the combined immunosuppressive regimen group

	Patient 1	Patient 2	Patient 3	Patient 4
Diagnosis	DM	CADM	DM	DM
Sex	M	M	F	M
Age, years	36	61	55	68
Survival date (days)	177	210	84	140
Exacerbation of ILD	(+)	(+)	(+)	(+)
Diabetes mellitus	(+)	(+)	(-)	(+)
Renal disorder*	(+) (HD)	(-)	(+)	(-)
CMV reactivation	(-)	(+)	(+)	(+)
TMP/SMZ prophylaxis	(-)	(-)	(+)	(+)
PCP	(+)	(+)	(-)	(-)
Other events	Pyothorax ILD recurrent	Iliopsoas abscess	Sepsis (MRCNS)	Sepsis (Lactobacillus) Meningitis
Anti-MDA5 (0 w, U/mL)	192.1	231.3	179.1	253.0
Anti-MDA5 (on death)	7.9	23.5	14.9	10.3
Ferritin (0 w, ng/mL)	3353.5	4236.0	237.4	374.4
Ferritin (on death)	15488.0	2454.0	1597.6	201.6

DM, dermatomyositis; CADM, clinically amyopathic dermatomyositis; ILD, interstitial lung disease; HD, hemodialysis; CMV, cytomegalovirus; TMP/SMZ, Trimethoprim-sulfamethoxazole; PCP, pneumocystis pneumonia; MRCNS, methicillin-resistant coagulase-negative staphylococci; MDA5, melanoma differentiation-associated gene 5.

*Renal disorder was defined as an increase in serum creatinine to 1.5-fold greater than initial level.

Supplementary Table 4

Infections that occurred during the 52-week follow-up period in the step-up treatment group

	Surviving patients (n = 5), n, %	Deceased patients (n = 10), n, %	P value
Bacterial infection	2 (40%)	3 (30%)	1
CMV reactivation	1 (20%)	4 (40%)	0.6
HSV/VZV	1 (20%)	1 (10%)	1
Candidiasis	2 (40%)	3 (30%)	1
Aspergillus	0 (0%)	0 (0%)	--
PCP	0 (0%)	2 (20%)	0.52

CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus; PCP, pneumocystis pneumonia.

Analysis was performed by Fisher's exact test.

Supplementary Table 5 Clinical features at the time of enrollment compared between patients who received combined immunosuppressive regimen with/without plasmapheresis and patients who received combined immunosuppressive regimen without plasmapheresis

	Prospective regimen group: Combined immunosuppressive regimen with/without plasmapheresis (n = 29)	Control group B: Combined immunosuppressive regimen without plasmapheresis (n = 17)	P value
Diagnosis	DM: 14 (48%) CADM: 15 (52%)	DM: 8 (47%) CADM: 9 (53%)	1
Onset type*	Acute: 8 (30%) Subacute: 15 (56%) Chronic: 4 (15%)	Acute: 5 (17%) Subacute: 9 (53%) Chronic: 3 (18%)	1
Female sex	19 (66%)	15 (88%)	1
Age, years	53.4 ± 13.5	48.9 ± 12.99	0.32
BMI	21.3 ± 3.06	20.9 ± 2.79	0.60
Heliotrope rash	13 (46%)	3 (19%)	0.16
Gottron's sign	28 (97%)	16 (94%)	1
Skin ulcer	7 (24%)	4 (25%)	0.55
Proximal muscle weakness	13 (46%)	4 (24%)	0.25
CK (U/L)	284.7 ± 324.6	223.9 ± 148.0	0.99
Ferritin (ng/mL)	689.7 ± 973.9	535.9 ± 572.6	0.72
LDH (U/L)	390.6 ± 138.9	390.5± 169.2	0.59
CRE (mg/dL)	0.58 ± 0.11	0.54 ± 0.12	0.25
CRP (mg/dL)	1.47 ± 2.04	0.76 ± 1.21	0.56
ESR (mm/hour)	44.8 ± 22.9	33.6 ± 16.1	0.17
KL-6 (U/mL)	689.7 ± 973.9	509.5± 143.8	0.08
SP-D (ng/mL)	64.6 ± 57.2	43.6 ± 21.6	0.51
PaO_2 (mmHg)	80.3 ± 13.9	79.8 ± 17.5	0.39
%VC (%)	78.8 ± 16.1	78.3 ± 26.5	0.86
FEV_1/FVC (%)	82.6 ± 8.40	78.2± 20.8	0.32
DLco (mL/min/mmHg)	14.0 ± 3.82	13.9 ± 2.50	0.79
DLco (%)	62.0 ± 14.9	64.3 ± 10.8	0.62

DM, dermatomyositis; CADM, clinically amyopathic dermatomyositis; CK, creatinine kinase; LDH, lactate dehydrogenase; CRE, creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; KL-6, Krebs von den Lungen-6; SP-D, pulmonary surfactant protein D; PaO_2, partial pressure of arterial oxygen; MDA5, melanoma differentiation-associated gene 5; %VC, percent vital capacity; FEV_1/FVC, forced expiratory volume in 1 second; DLco, diffusing capacity for carbon monoxide.

*Onset type was classified into acute (within 1 month), subacute (1 to 3 months), and chronic (over 3 months). Data were expressed as mean ± standard deviation for variables. Analysis was performed by either Mann–Whitney U test for continuous variables or Fisher's exact test for nominal variables.

Supplementary Table 6

Comparison of treatment between patients who received combined immunosuppressive regimen with/without plasmapheresis and patients who received combined immunosuppressive regimen without plasmapheresis

Treatment	Prospective regimen group: Combined immunosuppressive regimen with/without plasmapheresis (n = 29)	Control group B: Combined immunosuppressive regimen without plasmapheresis (n = 17)	P value
PSL	29 (100%)	17 (100%)	--
PSL (mg/day)	56.5 ± 10.3	54.7 ± 7.6	0.49
IVCY	29 (100%)	17 (100%)	--
Calcineurin inhibitors	29 (100%)	17 (100%)	--

PSL, prednisolone; IVCY, intravenous cyclophosphamide.

Analysis was performed by either Mann–Whitney U test for continuous variables or Fisher's exact test for nominal variables.

Supplementary Table 7

Comparison of periods of drug initiation between patients who received combined immunosuppressive regimen with/without plasmapheresis and patients who received combined immunosuppressive regimen without plasmapheresis

Period	Drugs to start	Prospective regimen group: Combined immunosuppressive regimen with/without plasmapheresis (n = 29)	Control group B: Combined immunosuppressive regimen without plasmapheresis (n = 17)	P value
From admission to hospital (days)	GC	3.3 ± 5.3	1.1 ± 1.9	0.14
	IVCY	5.7 ± 5.5	3.6 ± 4.9	0.12
	Calcineurin inhibitors	3.8 ± 5.5	1.1 ± 1.9	0.052
From onset (days)	GC	93.4 ± 50.1	65.2 ± 40.4	0.03
	IVCY	100.7 ± 47.2	67.9 ± 40.6	0.01
	Calcineurin inhibitors	98.0 ± 47.5	65.2 ± 40.4	0.01

GC, glucocorticoid; IVCY, intravenous cyclophosphamide.
Analysis was performed by Mann–Whitney U test.

Supplementary Table 8

Comparison between surviving and deceased patients in the combined immunosuppressive regimen group

	Surviving patients (n = 23), n, %	Deceased patients (n = 4), n, %	P value
Diagnosis	DM: 10 (43%) CADM: 13 (57%) Acute: 4 (19%)	DM: 3 (75%) CADM: 1 (25%) Acute: 2 (50%)	0.33
Onset type*	Subacute: 14 (67%) Chronic: 3 (14%)	Subacute: 1 (25%) Chronic: 1 (25%)	--
Female sex	16 (70%)	1 (25%)	0.13
Age, years	51.5 ± 13.1	55.0 ± 11.9	0.68
Heliotrope rash	11 (50%)	2 (50%)	1
Gottron's sign	22 (96%)	4 (100%)	1
Cutaneous ulcer	3 (13%)	3 (75%)	0.02
Proximal muscle weakness	9 (41%)	3 (75%)	0.31
Fever	15 (65%)	3 (75%)	1
CK (IU/L)	219.3 ± 236.4	644.3 ± 474.2	0.028
Ferritin (ng/mL)	459.9 ± 516.3	2050.3 ± 1772.7	0.16
LDH (U/L)	363.7 ± 126.3	535.3 ± 144.5	0.048
γGTP (U/L)	41.4 ± 45.6	139.5 ± 146.3	0.43
CRE (mg/dL)	0.57 ± 0.1	0.58 ± 0.06	0.81
CRP (mg/dL)	0.77 ± 1.2	2.7 ± 1.1	0.01
ESR (mm/hour)	41.4 ± 24.0	57.0 ± 7.9	0.32
HbA1c (%)	6.0 ± 0.33	6.5 ± 0.33	0.04
KL-6 (U/mL)	671.7 ± 346.0	738.6 ± 383.6	0.92
SP-D (ng/mL)	70.3 ± 61.3	40.4 ± 25.9	0.22
PaO_2 (mmHg)	81.4 ± 14.9	75.1 ± 3.1	0.44
Anti-MDA5 (U/mL)	210.0 ± 44.9	213.9 ± 30.0	0.92
%VC (%)	80.9 ± 15.9	74.2 ± 9.1	0.21
FEV_1/FVC (%)	83.9 ± 7.9	78.4 ± 0.84	0.21
DLco (mL/min/mmHg)	16.5 ± 11.1	14.8 ± 3.8	0.90
DLco (%)	61.2 ± 13.4	69.0 ± 30.5	0.90
Plasmapheresis	5 (22%)	4 (100%)	0.007
Periods from onset to start treatment (days)	95.6 ± 46.9	93.0 ± 65.8	0.66
Periods from hospitalization to start treatment (days)	3.32 ± 5.39	0.50 ± 0.87	0.22
Total HRCT score	9.95 ± 6.75	14.7 ± 4.33	0.13
Airspace consolidation	3.24 ± 2.93	6.75 ± 4.90	0.24
Ground-glass attenuation	4.53 ± 3.14	5.33 ± 1.85	0.39
Interlobular septal thickening and/or reticular opacity	2.17 ± 3.15	2.58 ± 2.71	0.45

DM, dermatomyositis; CADM, clinically amyopathic dermatomyositis; CK, creatinine kinase; LDH, lactate dehydrogenase; γGTP, gamma-glutamyl transpeptidase; CRE, creatinine; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HbA1c, glycated hemoglobin; KL-6, Krebs von den Lungen-6; SP-D, pulmonary surfactant protein D; PaO_2, partial pressure of arterial oxygen; MDA5, melanoma differentiation-associated gene 5; %VC, percent vital capacity; FEV_1/FVC, forced expiratory volume in 1 second; DLco, diffusing capacity for carbon monoxide; HRCT, high-resolution computed tomography.

*Onset type was classified into acute (within 1 month), subacute (1 to 3 months), and chronic (over 3 months). Data were expressed as mean ± standard deviation for variables. Analysis was performed by either Mann–Whitney U test for continuous variables or Fisher's exact test for nominal variables.

Supplementary Table 9

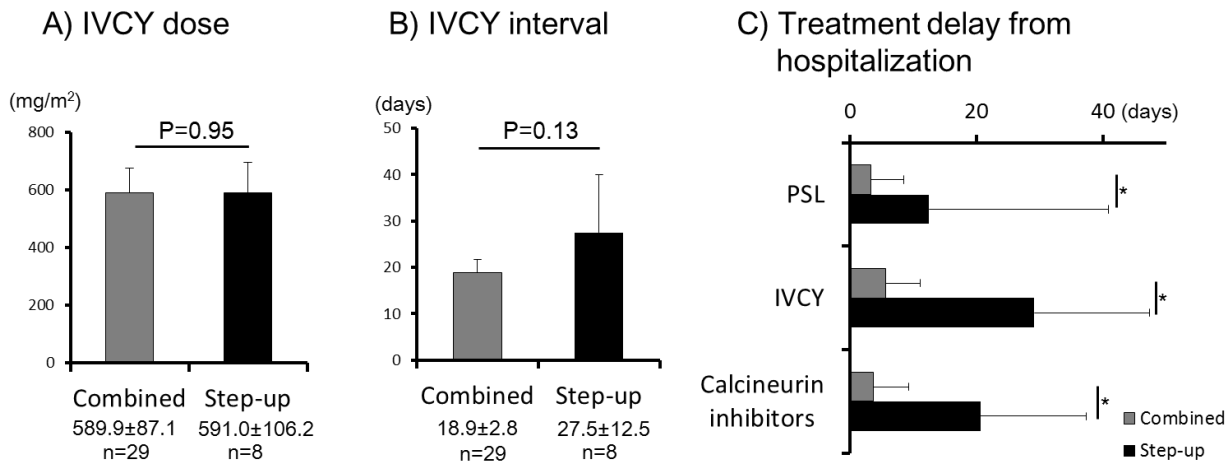
Comparison of mean blood tacrolimus concentrations (ng/mL) based on adverse events during the 52-week follow-up period in the combined immunosuppressive regimen group

	Adverse events		P value
	(-)	(+)	
Infection (total)	8.28 ± 2.42	7.11 ± 1.55	0.44
Bacterial infection	7.16 ± 1.78	7.75 ± 1.68	0.27
CMV	8.20 ± 2.43	7.13 ± 1.56	0.61
HSV/VZV	7.22 ± 1.71	9.43	0.13
Candidiasis	7.32 ± 1.25	7.33 ± 2.11	0.95
Aspergillus	7.22 ± 1.71	9.43	0.13
PCP	7.30 ± 1.77	7.72	0.81
Other fungal infections	7.32 ± 1.73	--	--
Diabetes mellitus	6.37 ± 1.32	7.76 ± 1.76	0.07
Hyperglycemia	6.48 ± 1.41	7.63 ± 1.78	0.18
Hyperlipidemia	7.92 ± 1.74	7.23 ± 1.76	0.32
Insomnia	8.52 ± 2.08	6.97 ± 1.51	0.09
Compression fracture	7.47 ± 1.63	4.26	0.10
Femoral head necrosis	7.27 ± 1.76	8.32	0.39
Hypertension	7.05 ± 1.47	7.90 ± 2.21	0.38
TMA	7.32 ± 1.73	--	--
Hyponatremia	7.60 ± 1.14	7.24 ± 1.89	0.51
Hypokalemia	7.65 ± 0.91	6.92 ± 2.38	0.21
Hyperkalemia	6.70 ± 1.84	8.40 ± 0.77	0.008

CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus; PCP, pneumocystis pneumonia; TMA, thrombotic microangiopathy.
Analysis was performed by Mann–Whitney U test.

Supplementary Figure 1

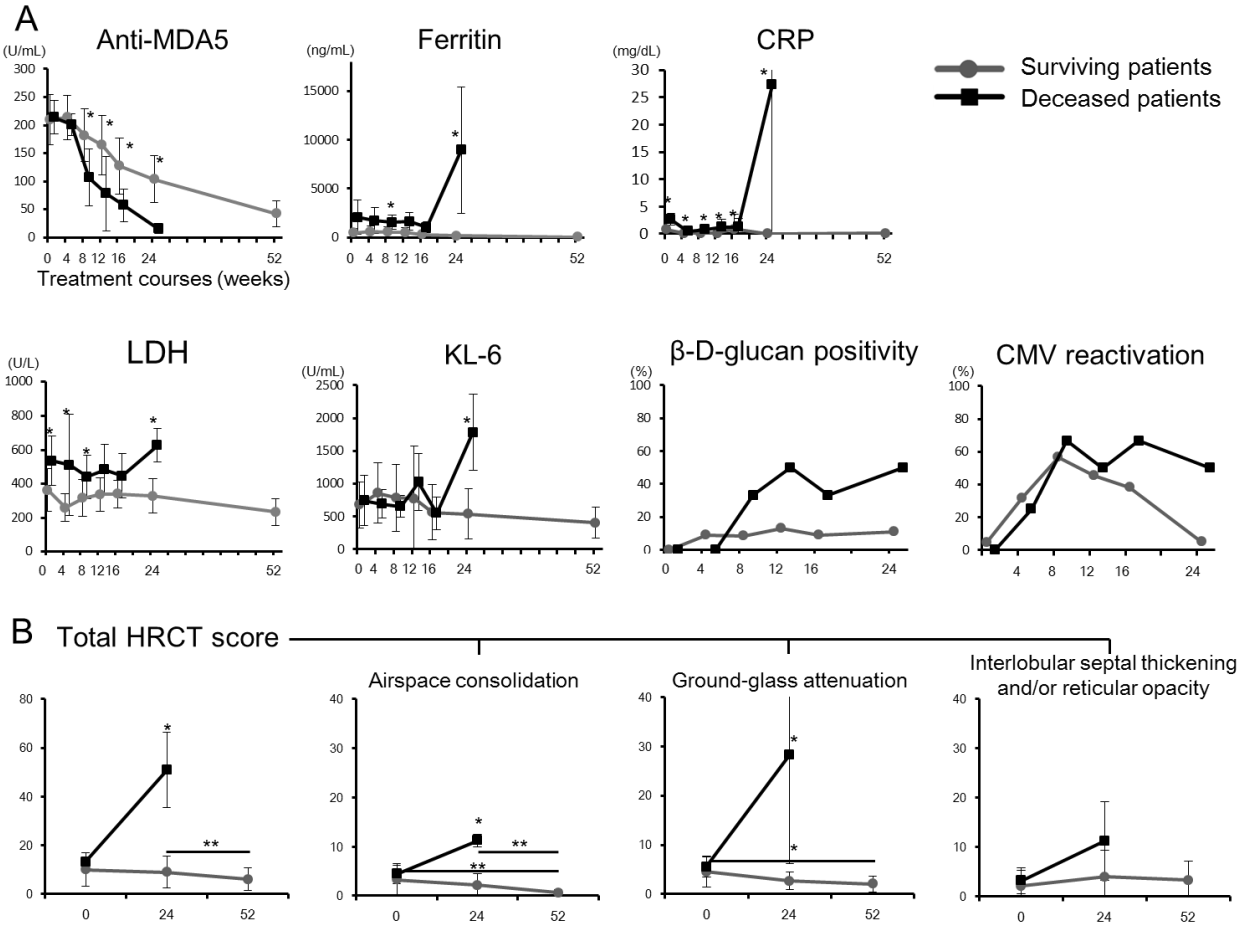
Doses and intervals of intravenous cyclophosphamide and periods to start medications



In intravenous cyclophosphamide (IVCY)-treated cases, the IVCY dose (A) and interval of IVCY treatment (B) were compared between the combined immunosuppressive regimen group (prospective regimen group, gray bar) and the step-up treatment group (control group A, black bar). The treatment delay of each drug was compared between the two groups (C). All data are shown as mean ± SD. Mann–Whitney U test, * $P < 0.05$

Supplementary Figure 2

Comparison of laboratory data and computed tomography scores between surviving and deceased patients.

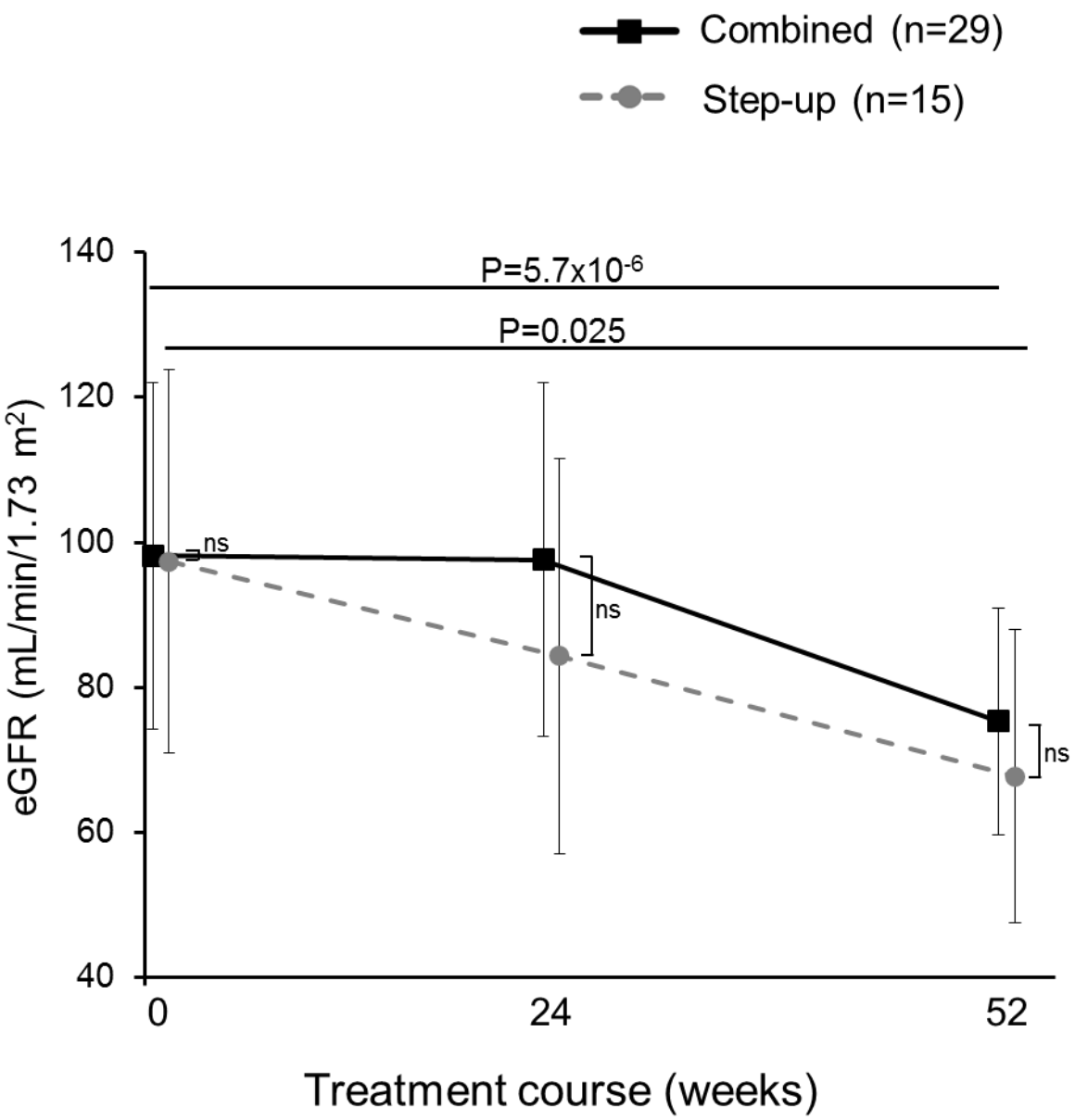


Line graphs show mean ± standard deviation of laboratory data (A) and chest high-resolution computed tomography (HRCT) scores (B) during the treatment course. Data were compared between surviving patients (gray line) and deceased patients (black line) at the same time point. The Mann–Whitney U test was used for continuous variables and Fisher's exact test was used for nominal variables. HRCT scores were also compared between different time points in the same group by using paired Student's *t*-test, where p value was adjusted by Bonferroni's method (B). *indicates $P < 0.05$, **indicates $P < 0.01$.

MDA5, melanoma differentiation–associated gene 5; CRP, C-reactive protein; LDH, lactate dehydrogenase; KL-6, Krebs von den Lungen-6; CMV, cytomegalovirus.

Supplementary Figure 3

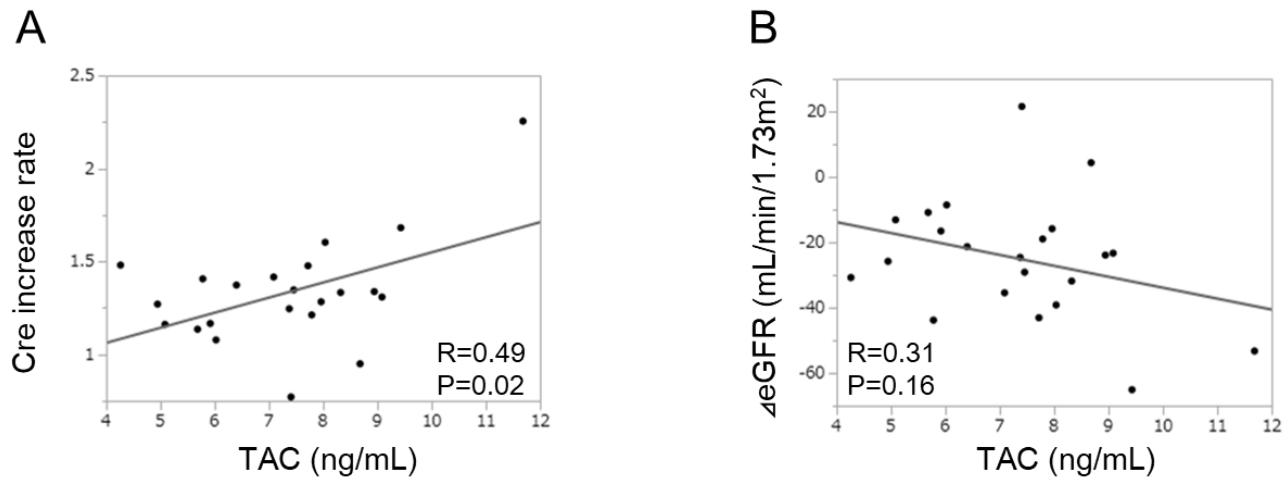
Comparison of renal function between the combined immunosuppressive regimen and the step-up treatment groups during treatment courses



Line graphs show mean ± standard deviation of eGFR in the combined immunosuppressive regimen group (prospective regimen group, solid line, n = 29) and the step-up treatment group (control group A, dotted line, n = 15) during the treatment course. Data were compared between the combined immunosuppressive therapy and the step-up treatment groups at the same time point by using the Mann–Whitney U test. Data were also compared between different time points in the same group by using paired Student's *t*-test. ns indicates not significant.

Supplementary Figure 4

Correlations between mean blood concentration of tacrolimus and alteration of renal function during the 52-week follow-up period in the combined immunosuppressive regimen group



Graphs show correlations between mean tacrolimus (TAC) blood concentration and the increase in serum creatinine (Cre) level (A) and between mean TAC blood concentration and alteration of estimated glomerular flow rate (eGFR) (B) during the 52-week follow-up period in the combined immunosuppressive regimen group. R represents Pearson's correlation coefficient.