Long term prognosis of anti-melanoma differentiation-associated gene 5-positive dermatomyositis with interstitial lung disease

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The authors declare no conflicts of interest.

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Abstract

Objective: Anti-melanoma differentiation-associated gene 5 (MDA5)-positive dermatomyositis with interstitial lung disease (DM-ILD) progresses rapidly and has a poor prognosis. Recently, we reported the efficacy of a combination therapy comprising high-dose glucocorticoids (GCs), calcineurin inhibitors (CNIs), and intravenous cyclophosphamide (IVCY) in a multicenter clinical trial (UMIN000014344). In the present study, we evaluated the long-term outcomes and effects of induction therapy on the maintenance of remission.

Methods: All participants from our previous trial were followed-up for more than 5 years. Seventy-three other patients with anti-MDA5-positive DM-ILD from our institute were retrospectively integrated into the previous trial for further analysis. Sixty-eight patients achieved remission and survived for >6 months. Based on the induction treatment, we classified the patients into 2 groups: group T (n=56), triple combination therapy (GCs, CNIs, and IVCY) and group C (n=12), mono/dual-therapy. The recurrence-free and drug-withdrawal rates of immunosuppressive agents were compared.

Results: The overall survival and recurrence-free survival rates at 5 years were 100% for the participants in the previous trial. The 5-year cumulative withdrawal rates for CNIs and GCs were 70% and 53%, respectively. In a comprehensive analysis, the recurrence-

55 free rates in group T were higher than those in group C (90% vs. 56%, P<0.05). The drug-
56 withdrawal rates of CNIs and GCs at 10 years in group T were also higher than those in
57 group C (79% vs. 0%, 43% vs. 0%, respectively, P<0.05).
58 **Conclusion:** Triple combination therapy in the induction phase can reduce the risk of
59 recurrence and facilitate drug withdrawal in anti-MDA5-positive DM-ILD.

Introduction

Clinically, amyopathic dermatomyositis (CADM) is a disease with the typical skin manifestations of dermatomyositis (DM), with few or no features of myopathy (1, 2). The anti-melanoma differentiation-associated gene 5 (MDA5) antibody is strongly associated with CADM (3, 4). Interstitial lung disease (ILD) accompanied by anti-MDA5-positive DM/CADM is often rapidly progressive and associated with a poor prognosis, particularly in Japanese patients (5, 6). In the past, approximately 50% of patients died from ILD exacerbation within 6 months of presentation (7). The mortality seemed to be due to the insufficiency of immunosuppressants, such as the administration of only glucocorticoids (GCs) or GCs and calcineurin inhibitors (CNIs).

Recently, we reported the efficacy of combined immunosuppressive therapy comprising high-dose GCs, CNIs, and intravenous cyclophosphamide (IVCY) for anti-MDA5-positive DM/CADM-ILD in a single-arm prospective trial (8). With this triple-combination therapy, the 6-month survival rates improved to 89% compared with those with conventional therapy (8). The efficacy of plasma exchange (PE) as an additional therapy for anti-MDA5-positive DM/CADM has also been reported (9). These triple combination therapies and additional PE have improved survival rates and prolonged survival.

In accordance with the improvement in the survival rate associated with induction therapy, more patients that are anti-MDA5-positive can be followed-up for a longer time. However, no study, to our knowledge, has evaluated long-term maintenance therapy for anti-MDA5-positive DM/CADM-ILD and the management of patients who are anti-MDA5-positive in remission.

In this study, long-term follow-up data for participants from our previous multicenter, single-arm clinical trial (UMIN000014344) (8) were analyzed. Subsequently, by combining the participants of the clinical trial with another cohort, we retrospectively investigated the effect of the induction therapy on long-term outcomes and the changes in maintenance immunosuppressive therapy for anti-MDA5-positive DM/CADM-ILD.

Methods

Study overview

First, we analyzed the long-term outcomes and maintenance therapy of all participants from our previous trial (UMIN000014344) (8) who were followed-up for more than 5 years. Eligible patients were anti-MDA5 positive and diagnosed as having definite, probable, or possible DM or CADM with ILD, without immunosuppressive treatment before admission to one of the registered hospitals, and who were followed-up

for more than 5 years from initial therapy by March 2022. Patients with the following conditions were excluded: deceased patients who did not achieve remission and patients followed-up for less than 6 months from initial therapy. DM was diagnosed according to the criteria of Bohan and Peter (10). CADM was diagnosed when patients demonstrated typical cutaneous lesions of DM without clinical evidence of myositis, with minimal or no increase in serum creatine kinase (2, 10, 11). ILD diagnosis was based on respiratory symptoms, physical examination, high-resolution chest computed tomography (CT) findings, and respiratory function tests. Serum anti-MDA5 antibodies were detected by immunoprecipitation using ^{35}S-labeled HeLa cells and enzyme-linked immunosorbent assay (MESACUP anti-MDA5 test, MBL Co., Ltd., Tokyo, Japan) (4, 12). Remission was defined as survival for >6 months after initial treatment without active exacerbation of ILD.

Second, to investigate the influence of induction therapy on long-term outcomes and management, we retrospectively enrolled 73 consecutive Japanese patients with anti-MDA5-positive DM/CADM-ILD who were aged ≥18 years and had visited or been referred to Kyoto University Hospital from October 2001 to March 2022. We combined them with the participants of the clinical trial and analyzed prognosis, such as recurrence-free rate or drug-withdrawal rate. Inclusion criteria were as follows: Japanese patients

aged ≥18 years with anti-MDA5-positive DM/CADM-ILD who had achieved remission and survived without relapse of ILD. The criteria of DM, CADM, ILD, and remission and the measuring method for anti-MDA5 antibodies were as described above. Relapse of ILD was defined as a deterioration in the respiratory function test or new ground-glass opacities on chest CT, assessed by both a radiologist and a rheumatologist, and the requirement for intensified treatment (13).

Patients with the following conditions were excluded: deceased patients who could not achieve remission, patients who had received initial therapy mainly at another hospital, patients who had been followed-up for less than 6 months from initial therapy, patients who had been diagnosed with malignancy at the same time, and patients who had other myositis-specific autoantibodies, such as anti-aminoacyl-transfer ribonucleic acid synthetase antibodies. Eligible patients for combined analysis are shown in Figure 1. We excluded 28 patients, including 17 who were not in remission and died less than 6 months after the initial therapy, 6 who received undetermined induction therapy at another hospital, 3 for whom remission could not be confirmed because the follow-up time was less than 6 months, 1 who was diagnosed with malignant lymphoma just after remission of lung disease, and 1 who was positive for both anti-ARS and anti-MDA5 antibodies.

There were 68 Japanese adult patients with anti-MDA5-positive DM/CADM-

ILD who achieved remission and survived for >6 months. These patients were divided into Group T, comprising patients treated with triple combination therapy (GCs+CNIs+IVCY) (n=56) and group C, comprising patients treated with conventional therapy (only GCs, GCs+CNIs, or GCs+IVCY) (n=12), although the treatment regimen was decided on by each attending physician at the time. Furthermore, to investigate the effect of early initial IVCY, patients in group T were subdivided into early group T and late group T, based on the timing of IVCY initiation.

All patients provided written informed consent to participate in this study prior to sample collection. The study was conducted in accordance with the Declaration of Helsinki. The study design was approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine (approval number; R1540).

Triple combination therapy (group T)

The triple combination therapy regimen comprised high-dose GCs, CNIs, and IVCY as remission induction agents. The regimen was initiated soon after or up to 3 months after the diagnosis of DM or CADM with ILD. Prednisolone was used as the main GC and initially administered at 1 mg/kg/day for 4 weeks; thereafter, the existing dose was reduced by 10% every 2 weeks. Tacrolimus or cyclosporine A was used as a CNI.

Tacrolimus was adjusted to maintain a 12-hour blood trough level 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 dose of 1,000 mg/m^2 of BSA according to a nadir leukocyte count from baseline. The intended number of IVCY administrations was 10-15. Patients who received initial IVCY within 7 days after GC administration were categorized into the early group T (n=45), whereas patients who received initial IVCY later than 7 days after GC administration were categorized into the late group T (n=11).

Conventional therapy (group C)

Group C patients received monotherapy (only GCs) or dual therapy (GCs with CNIs or IVCY) as remission induction therapy. Although methods of using GCs, CNIs, and IVCY have not been strictly defined, we defined “late addition of CNIs” as when patients had CNIs added after 3 months from GC administration. Other immunosuppressants were not strictly restricted at initial therapy.

The choice of treatment regimen was dependent on the attending physicians. In both groups, patients underwent PE as additional therapy when they worsened clinically and required oxygen administration during remission induction therapy.

168 ***End points***

169 The primary endpoint was the comparison of the recurrence-free rate at 120
170 months after the initial therapy between groups T and C. Recurrence-free time was
171 defined as the interval between the initiation of induction therapy for lung disease and the
172 exacerbation of lung disease or the last follow-up. Secondary endpoints included the
173 drug-free rate of GCs and/or CNIs at 60 or 120 months from initial therapy and the median
174 prednisolone dose at 36 months after initial induction therapy. We defined drug-free as
175 complete withdrawal of GCs and/or CNIs (temporal withdrawal of drugs was not defined
176 as drug-free). We also collected the clinical data of remission, adjusting for the time from
177 initial therapy and serious adverse events after initial therapy. Serious adverse events were
178 defined as unexpected admissions, such as infection or emergent surgery. We also
179 analyzed the differences in these end points among the 3 groups.

180

181 ***Statistics***

182 Recurrence-free and drug-free rates were estimated by Kaplan–Meier analysis.
183 The log-rank test was also used for comparison. The Wilcoxon test and Fisher's exact test
184 were used to assess the associations of clinical features between the groups. A P-value of
185 <0.05 was considered statistically significant. The P-values for multiple comparisons

were adjusted using Bonferroni correction. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) for R (The R Foundation for Statistical Computing, Vienna, Austria) (14).

Results

Long-term follow-up data of the clinical trial

The overall survival and recurrence-free survival rates of the participants who achieved remission in our previous multicenter, single-arm clinical trial (UMIN000014344) (9) are demonstrated in Figure 2A. Twenty-three patients were enrolled and all patients survived without relapse of ILD. The overall survival and recurrence-free survival rates at 60 months after the initial therapy were both 100% (the overall survival rate is not shown in the figure). Drug withdrawal rates of immunosuppressants in these patients are exhibited in Figure 2B-2D. The 60-month cumulative withdrawal rates for CNIs and GCs were 70% and 53%, respectively. The cumulative achievement rate for being drug-free of both CNIs and GCs at 60 months was 38%. The changes in clinical parameters from the induction phase to the remission phase are shown in Table 1. The median time of analyzing clinical remission was 2,330 days from initial therapy. Respiratory function and Krebs von den Lungen 6 (KL-6) were

204 significantly improved in the remission phase. Disease activity markers, such as serum
205 ferritin, KL-6, and anti-MDA5 antibody titers, improved 12 months after the induction of
206 initial therapy and remained within the normal range during the remission phase
207 (Supplementary Figure S1). All patients enrolled in our previous trial maintained
208 remission and had improved disease activity markers and respiratory functions.
209
210 ***Characteristics of patients for the combined retrospective analysis***
211 A comparison of the clinical characteristics and laboratory tests between the
212 groups is displayed in Table 2. Fifty-six patients were treated with triple combination
213 therapy (group T) and 12 patients with conventional therapy (group C). There were no
214 significant differences in age or sex between the 2 groups. Although the time from
215 disease-onset to initial treatment was significantly longer in group C ($P<0.001$), the
216 severity of ILD, such as saturation of peripheral oxygen and oxygen therapy at initial
217 therapy, was not different between the 2 groups. Serum ferritin and KL-6 levels were
218 elevated in both groups, with no significant differences between the groups.
219 The predicted percent of diffusing capacity for carbon monoxide (%DLco) was
220 significantly lower in group C than in group T ($P<0.01$). However, the predicted percent
221 of forced vital capacity did not differ between the groups. GCs were used for all patients

in both the groups and the initial median dose of prednisolone was significantly higher in group T ($P<0.01$). CNIs were used for all patients in group T, and not all patients in group C were treated with triple combination therapy due to the protocol. CNIs were started at approximately the same time as the GCs in all patients in group T, which was significantly earlier than group C. Immunosuppressants other than GCs, CNIs, or IVCY were not used at initial therapy in either of the groups (data not shown). PE was used more frequently for patients in group T than in group C (18% vs. 0%), although this difference was not significant. The median follow-up time was not significantly different between the groups.

The clinical characteristics of the 3 groups (early group T, late group T, and group C) are shown in Supplementary Table S1. Group C had a significantly greater time from disease onset to initial therapy compared with the early and late group T ($P<0.05$, Bonferroni adjustment). The %DLco and initial median dose of prednisolone in group C were significantly lower compared to the early group T (both $P<0.01$, Bonferroni adjustment). The frequency of tacrolimus use, total dose, and IVCY count in group C was significantly lower than in both the early and late group T (both $P<0.01$, Bonferroni adjustment).

Recurrence-free rate of the patients in the combined retrospective analysis

The overall survival rates for groups T and C were both 100% (data not shown). The overall recurrence-free survival is exhibited in Figure 3A. The cumulative recurrence-free rates at 120 months were 90% and 56% in group T and group C, respectively; these were significantly different (log-rank test, P=0.021). All patients who required oxygenation during the induction phase in both groups recovered to a stage where supplemental oxygen could be withdrawn. Group T was divided into the early and late groups. The overall recurrence-free rates at 120 months from initial therapy of the early T, late T, and C groups were 92%, 83%, and 56%, respectively (Supplementary Figure S2). Although there were no significant differences (log-rank test, P=0.06), the early group T demonstrated the highest recurrence-free survival rate among the 3 groups.

In terms of clinical characteristics and laboratory tests performed during the remission phase, KL-6 was significantly higher in group C, although respiratory function tests were not significantly different between both groups. However, this is only for reference due to the small number of patients in group C (Supplementary Table S2).

Drug withdrawal rate of immunosuppressants

The cumulative withdrawal rates of immunosuppressants, as well as drug-free rates, are exhibited in Figure 3B-3D. The 60-month cumulative withdrawal rates of CNIs

were 59% and 0% in group T and group C, respectively, which were significantly different (log-rank test, $P<0.01$). Moreover, the 120-month cumulative withdrawal rate of CNIs in Group T was 79%. The 60-month cumulative withdrawal rates of GCs were 32% and 0% in group T and C, respectively, which were significantly different (log-rank test, $P<0.05$). The cumulative drug-free rates for GCs at the 120-month point were 43% and 0% in groups T and C, respectively. Although there was no significant difference ($P=0.13$), only the patients in group T achieved drug-free (withdrawal of both CNIs and GCs) remission (17% at 60 months and 36% at 120 months).

The cumulative withdrawal rates for CNIs or GCs and drug-free rates for the early T, late T, and C groups are demonstrated in Supplementary Figure S3. The 60-month cumulative withdrawal rate for CNIs in group T was 68%, which was significantly higher than that in group C ($P<0.001$, with Bonferroni adjustment). The 60-month cumulative withdrawal rate for GCs in the early group T was 38%, which was also significantly higher than that in group C ($P<0.05$, Bonferroni adjustment). The 60-month and 120-month drug-free rates in the early T and late T groups were 22% and 43%, respectively, which were the highest among the values for the 3 groups. However, the difference was not significant.

The maintenance dose of prednisolone at 36 months

The prednisolone dose required 36 months after the initial induction therapy is demonstrated in Figure 4. The median dose of prednisolone at 36 months in group T was significantly lower than that in group C (3.0 mg/day and 6.0 mg/day, respectively; $P<0.01$). The median doses of prednisolone at 36 months in the early T, late T, and C groups were 3.0 mg/day, 3.5 mg/day, and 6.0 mg/day, respectively (Supplementary Figure S4). The median dose of prednisolone in the early group T was significantly lower than that in group C ($P<0.05$, Bonferroni correction).

Details of relapsed patients

There were 2, 1, and 4 patients who experienced lung relapse in the early T, late T, and C groups, respectively. The detailed clinical course of the patients with lung relapse is shown in Supplementary Table S3. One patient in the early group T was newly diagnosed with breast cancer several months before the relapse of ILD, and another patient experienced ILD relapse approximately 1 year after tacrolimus discontinuation due to liver dysfunction. Most of the relapsed patients could be re-induced to remission by increasing GCs to moderate or high doses and restarting IVCY and/or CNI; however, 2 patients in group C experienced a second relapse of skin manifestations or ILD after re-

induction therapy.

Adverse events

During the observation period, there were 14 (10 patients) and 6 (3 patients) serious adverse events in group T and group C, respectively. Most of the events were bacterial or viral infections. There was no malignancy, such as leukemia, myelodysplastic syndromes, malignant lymphoma, and bladder cancer, which are thought to be induced by cyclophosphamide (Supplementary Table S4), in either group. Serious adverse events of the 3 groups (early group T, late group T, and group C) are shown in Supplementary Table S5.

Discussion

We previously reported the importance of early intervention with triple combination therapy for remission induction and 6-month survival of patients with anti-MDA5-positive DM-ILD in a multicenter prospective trial. However, there is no reported evidence regarding management during the remission phase (8). To our knowledge, this is the first study to demonstrate the effects of induction therapy on long-term outcomes or maintenance treatment for anti-MDA5-positive DM/CADM-ILD.

312 In the present study, the long-term survival and relapse-free rates of patients with
313 anti-MDA5-positive DM-ILD treated with triple combination therapy remained high.
314 Some patients achieved complete drug-free status after the withdrawal of both GCs and
315 CNIs. Disease monitoring markers of interstitial pneumonia, such as KL-6 and respiratory
316 function tests, demonstrated significant improvement 12 months after the initial treatment.
317 These improvements were maintained for an extended period of time. These findings
318 suggested that the efficacy of triple combination therapy, including IVCY, affected not
319 only survival in the remission induction phase but also disease stability with minimum
320 immunosuppressants agents in the maintenance phase. Based on these results, the present
321 study investigated whether the difference in remission induction therapies affects long-
322 term prognosis and stability during the maintenance phase in patients with anti-MDA5-
323 positive DM-ILD who survived the acute phase of their disease.

324 In order to collect data from and analyze patients with anti-MDA5-positive DM-
325 ILD who had achieved remission, we integrated another retrospective cohort of 73
326 patients from our institution into the previous clinical trial (8) and divided them into 2
327 groups for retrospective analysis, based on the remission induction treatment received.
328 There were no significant differences between the 2 groups in terms of age, lactate
329 dehydrogenase, C-reactive protein, and ferritin levels, which have been reported as poor

prognostic factors (15).

However, the time from onset to treatment was significantly longer in group C, and respiratory function tests, such as %DLco, were also significantly poorer in group C. Group C included more patients who were treated during the previous era than group T. This suggested that the diagnosis and initiation of treatment had been delayed, and interstitial pneumonia may have progressed due to the lack of established testing methods for anti-MDA5 antibodies. However, after remission, group C patients demonstrated improvement in respiratory function, which was not significantly different from that of group T (Supplementary Table S2). This suggested that the respiratory function of patients who are anti-MDA5-positive can be improved to some extent after remission induction, even if their ILD is somewhat advanced before treatment. Therefore, it is unlikely that differences in diagnosis-delay and respiratory function prior to induction therapy affected relapse or drug-withdrawal rates during long-term observation.

Some patients were treated with plasma exchange in group T, although there are conflicting reports of the effectiveness of PE (9, 16). PE in this study was only used in the induction phase and the number of patients treated with PE was low. Thus, this was not thought to influence the duration of remission achievement. Considering this, we do not believe that there were any major differences in patient background between the 2

groups in this study, other than differences in treatment protocols. Comparison between the 3 groups after subdividing group T into 2 groups exhibited the same trend.

The relapse-free rate was significantly higher for patients in Group T than in Group C. There was no significant difference between the rates in the early and late T groups. However, relapses in the early group T included patients who had discontinued immunosuppressive agents due to adverse effects and a patient who had experienced comorbid malignancy during the course of their disease (Supplementary Table S3). In contrast, relapses in the late group T and group C did not include patients with unintended treatment changes or coexisting malignancy. Therefore, early administration of a sufficient amount of cyclophosphamide might be beneficial for maintaining long-term remission.

The drug withdrawal rates were also significantly higher in group T than in group C. Although there was no statistical significance, a substantial number of group T patients achieved complete discontinuation of both GCs and CNIs, while none of the patients in group C achieved drug-free remission. The decision to withdraw drugs, such as GCs or immunosuppressive agents, was dependent on the discretion of the attending physician. Considering the medical record information, the attending physician decided to reduce or discontinue the drug based on the titer of anti-MDA5 antibodies, length of the relapse-

366 free period, and presence of clinical symptoms related to dermatomyositis, such as skin,
367 joint, and respiratory symptoms, although there is no pre-defined protocol for decreasing
368 and discontinuing drugs. Although the prednisolone dose was significantly higher in
369 group T than in group C during induction therapy, the prednisolone dose at 36 months
370 was significantly lower in group T. This suggests that the disease activity in group T was
371 stabilized earlier than that in group C, which resulted in a steady reduction in the GC dose.
372 When group T was further divided into 2 groups, patients who were able to discontinue
373 both GCs and CNIs were observed only in the early group T. This could be attributed to
374 the fact that triple combination therapy, including early IVCY, led to long-term deep
375 remission. About adverse events, although we did not compared statistically, there were
376 not seemed to be differences about serious adverse events in group T and group C.
377 Moreover, group T was treated a high total dose of cyclophosphamide, there were no
378 malignancies that could be induced by cyclophosphamide.

379 There have been several reports on the long-term prognosis of anti-MDA5-
380 positive DM-ILD. Isoda et al. (17) reported a significantly lower relapse rate in anti-
381 MDA5-positive DM-ILD than in anti-ARS-positive DM-ILD over a 2-year observation
382 period. With regards to long-term prognostic data, there are reports of a case involving
383 anti-MDA5-positive DM-ILD that remained in remission for approximately 7 years after

initial treatment and a case where ILD recurrence was only observed after 9 years (18, 19). However, these were relatively short-term studies with a small number of patients. Hence, these studies were insufficient to evaluate the long-term prognosis and management of anti-MDA5-positive DM-ILD during the remission period.

In our study, we included a substantial number of patients with anti-MDA5-positive DM-ILD, standardized the induction of remission therapy, and analyzed the data obtained from long-term observation (median: 5.0 years, range: 0.50-20.3 years). The survival and relapse-free rates of anti-MDA5-positive DM-ILD were clearly higher during the remission maintenance period in the initial triple combination therapy group. Even in relapse cases, re-induction therapy, including IVCY, demonstrated a high rate of remission re-induction.

This study had several limitations. First, there was selection bias regarding the inclusion of patients. We only analyzed patients who had survived for more than 6 months, and severe patients at initial therapy were excluded if they died within 6 months. Furthermore, patients in group C were diagnosed in the older era, so most patients had delayed diagnosis because the recently established commercial base method for detecting anti-MDA5 was not used. As a result, in this study we analyzed patients with generally mild disease activity. Second, this study included indication bias because this was a

retrospective study conducted by reviewing the charts and the management, including the prescription of drugs, dependent on the attending physician. Third, we only examined the activity of pulmonary lesions and did not assess other organ activities, such as skin or joint lesions. Since residual skin and joint lesions could affect the dose adjustment of GCs and CNIs, we preferred to evaluate overall disease activity using an established scoring system.

In conclusion, the induction of remission with triple combination therapy reduced the relapse risk of ILD and led to early dose reduction and discontinuation of immunosuppressive agents in the maintenance phase in many cases. Further investigation and validation are needed to establish a prospective study in which a drug-tapering protocol during the remission phase is defined and a multifaceted and standardized disease activity core set is used.

Author contributions

RN conceived the study. TS, HT, TN, YI, YY, and SH collected the clinical samples. TS analyzed the data and wrote the main manuscript. RN, HT, MS, RH, KK, SA, HY, TM, and AM contributed to the study design and manuscript writing. All authors have reviewed the manuscript and approved the final version for submission.

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421 **Data availability**

422 The data underlying this article will be shared on reasonable request to the

423 corresponding author.

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Figure legends

Figure 1.

FLOW SCHEME FOR THE ANALYSIS OF PATIENTS WITH ANTI-MDA5-POSITIVE DM-ILD WHO ACHIEVED REMISSION

Group T: Patients treated with GCs, CNIs, and IVCY.

Early group T: Patients who received initial IVCY within 7 days of GC administration.

Late group T: Patients who received initial IVCY within 3 months of GC administration.

Group C: Patients who received monotherapy (only GCs) or dual therapy (GCs with CNIs or GCs with IVCY).

DM, dermatomyositis; CADM, clinically amyopathic dermatomyositis; ILD, interstitial lung disease; MDA5, melanoma differentiation-associated gene 5; ARS, aminoacyl-tRNA synthetase; GCs, glucocorticoids; CNIs, calcineurin inhibitors; IVCY, intravenous cyclophosphamide

Figure 2.

CLINICAL COURSE OF PARTICIPANTS FROM THE PREVIOUS CLINICAL TRIAL

(A) Recurrence-free rate (B) Drug-free rate for calcineurin inhibitors (C) Drug-free rate for glucocorticoids (D) Drug-free rate for both calcineurin inhibitors and glucocorticoids

Twenty-three patients were enrolled. The cumulative rates were analyzed using the Kaplan–Meier test. GCs, glucocorticoids; CNIs, calcineurin inhibitors.

Figure 3.

CLINICAL COURSE OF PARTICIPANTS INCLUDED IN THE COMPREHENSIVE ANALYSIS

(A) Comparison of recurrence-free rates between group T (triple combination therapy group, n=56) and group C (conventional therapy group, n=12)

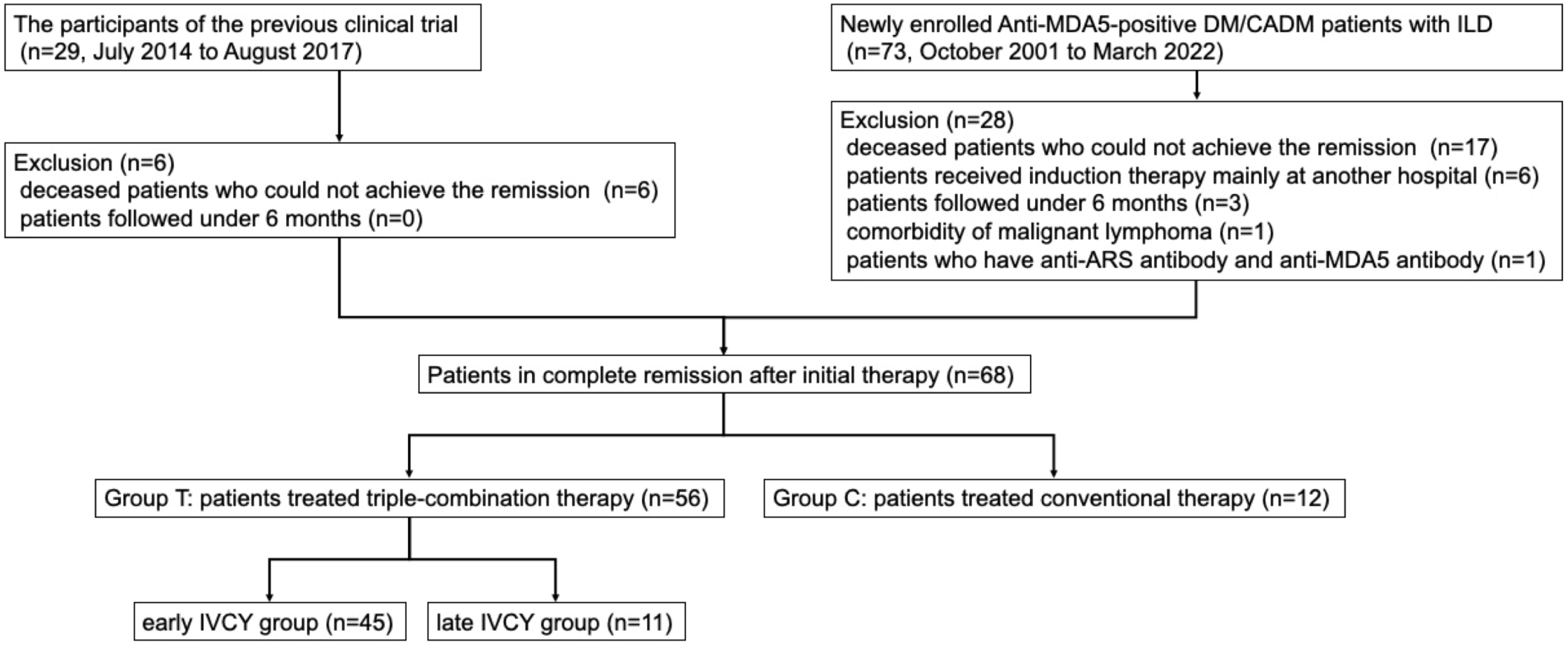
(B) Drug-free rate for calcineurin inhibitors

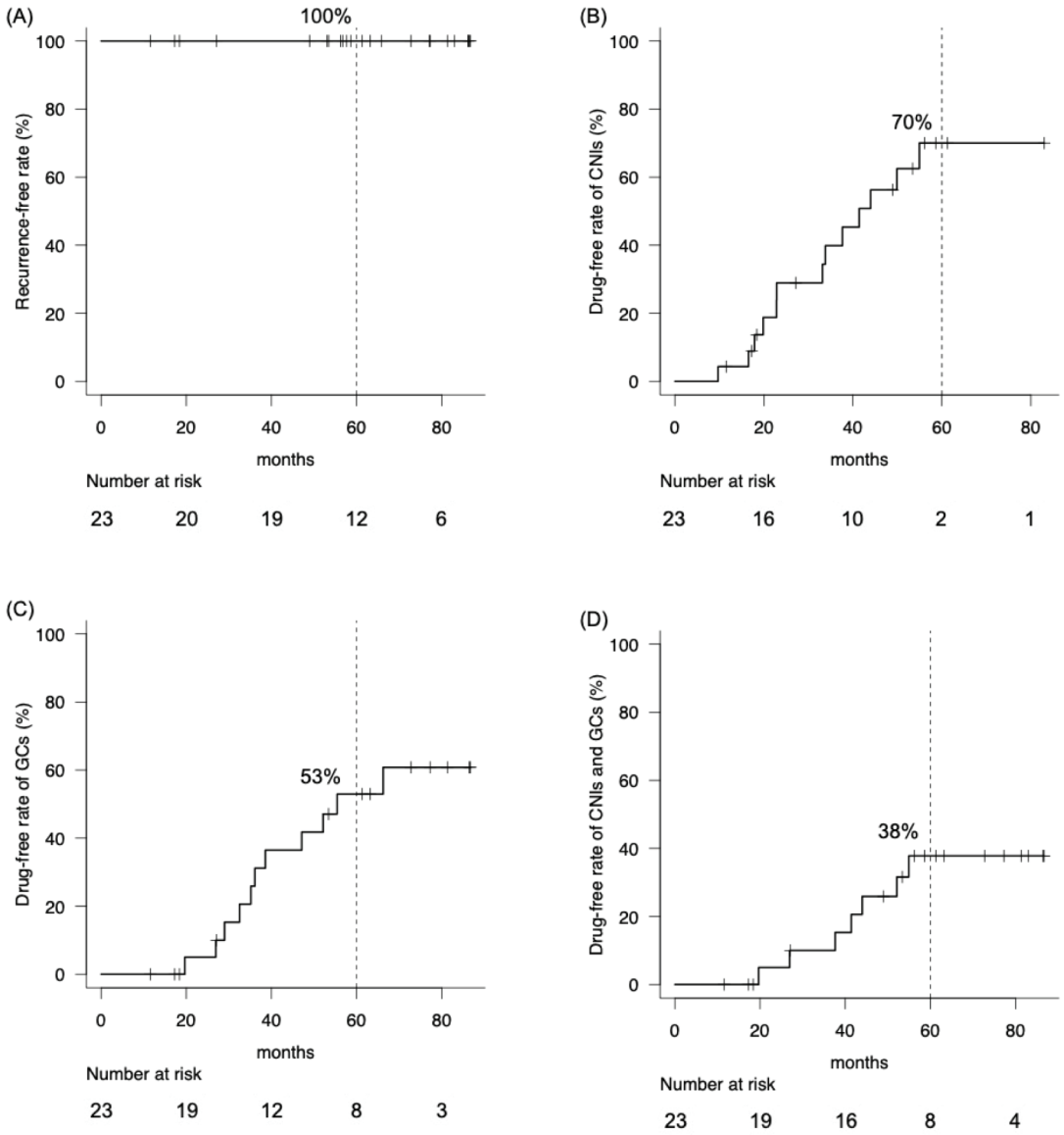
(C) Drug-free rate for glucocorticoids

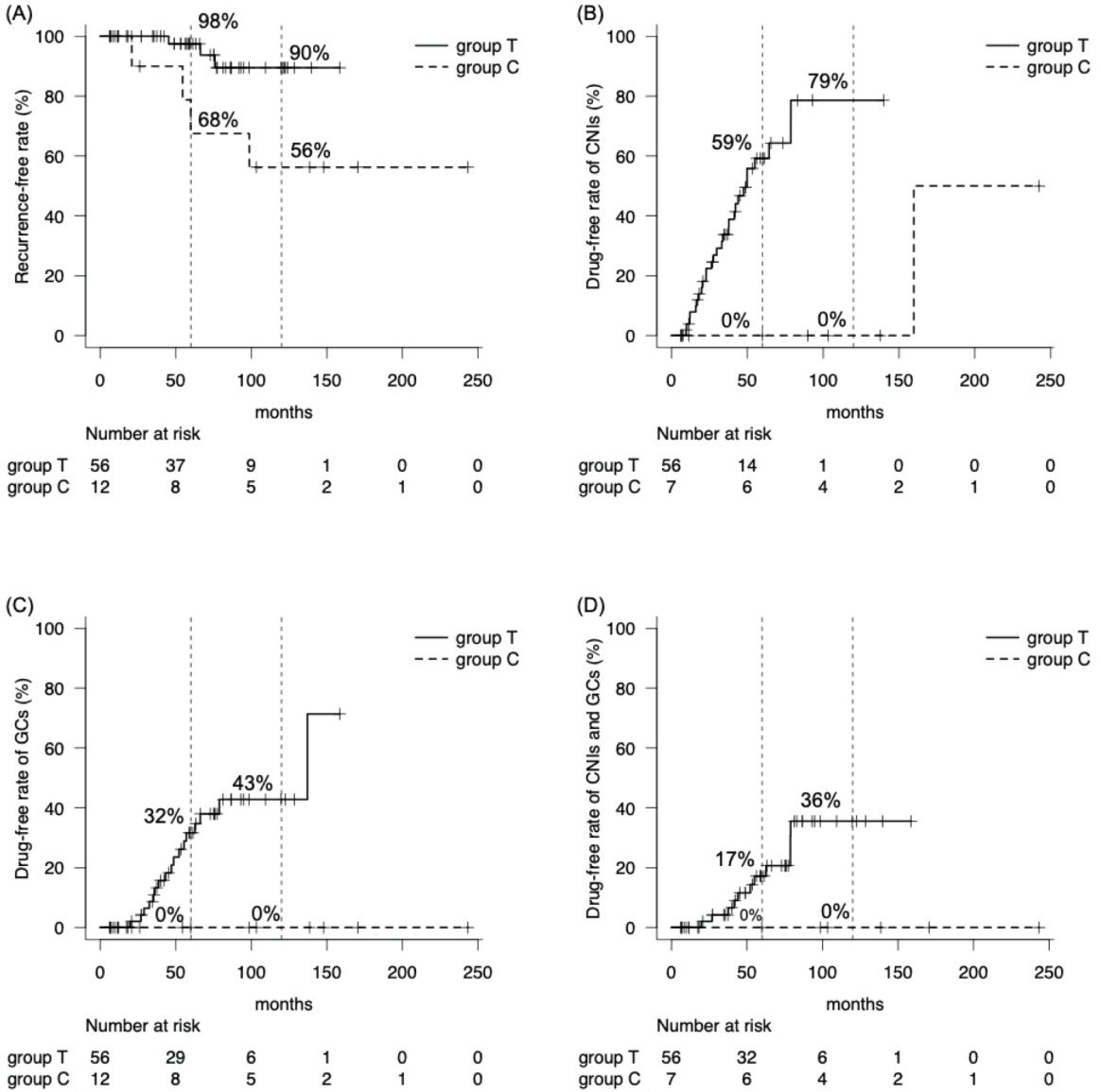
(D) Drug-free rate for both calcineurin inhibitors and glucocorticoids

The recurrence-free rate and drug-free rates for calcineurin inhibitors and glucocorticoids are significantly higher in group T than in group C ($P<0.05$, respectively). The cumulative rates were calculated using the Kaplan–Meier method.

521 The log-rank test was used to compare the rates. GCs, glucocorticoids; CNIs,
522 calcineurin inhibitors.
523
524 **Figure 4.**
525 **MAINTENANCE DOSE OF PREDNISOLONE AT 36 MONTHS AFTER**
526 **INDUCTION OF INITIAL THERAPY**
527 The median doses of prednisolone are 3.0 mg/day and 6.0 mg/day in group T (n=45)
528 and group C (n=8), respectively, which are significantly different based on the Mann–
529 Whitney U test ($P<0.01$). Data are presented as box plots, where the boxes represent the
530 25^{th} to 75^{th} percentiles, the lines within the boxes represent the median values, and the
531 lines outside the boxes represent the minimum and maximum values.
532


The participants of the previous clinical trial (n=29, July 2014 to August 2017)
Newly enrolled Anti-MDA5-positive DM/CADM patients with ILD (n=73, October 2001 to March 2022)
Exclusion (n=6)
deceased patients who could not achieve the remission (n=6)
patients followed under 6 months (n=0)
Exclusion (n=28)
deceased patients who could not achieve the remission (n=17)
patients received induction therapy mainly at another hospital (n=6)
patients followed under 6 months (n=3)
comorbidity of malignant lymphoma (n=1)
patients who have anti-ARS antibody and anti-MDA5 antibody (n=1)
Patients in complete remission after initial therapy (n=68)
Group T: patients treated triple-combination therapy (n=56)
Group C: patients treated conventional therapy (n=12)
early IVCY group (n=45)
late IVCY group (n=11)


(A)
100%
Recurrence-free rate (%)
100
80
60
40
20
0
0
20
40
60
80
months
Number at risk
23 20 19 12 6
(B)
70%
Drug-free rate of CNIs (%)
100
80
60
40
20
0
0
20
40
60
80
months
Number at risk
23 16 10 2 1
(C)
53%
Drug-free rate of GCs (%)
100
80
60
40
20
0
0
20
40
60
80
months
Number at risk
23 19 12 8 3
(D)
38%
Drug-free rate of CNIs and GCs (%)
100
80
60
40
20
0
0
20
40
60
80
months
Number at risk
23 19 16 8 4


(A)
Recurrence-free rate (%)
98%
90%
68%
56%
group T
group C
months
Number at risk
group T 56 37 9 1 0 0
group C 12 8 5 2 1 0
(B)
Drug-free rate of CNIs (%)
59%
79%
0%
0%
group T
group C
months
Number at risk
group T 56 14 1 0 0 0
group C 7 6 4 2 1 0
(C)
Drug-free rate of GCs (%)
32%
43%
0%
0%
group T
group C
months
Number at risk
group T 56 29 6 1 0 0
group C 12 8 5 2 1 0
(D)
Drug-free rate of CNIs and GCs (%)
17%
36%
0%
0%
group T
group C
months
Number at risk
group T 56 32 6 1 0 0
group C 7 6 4 2 1 0

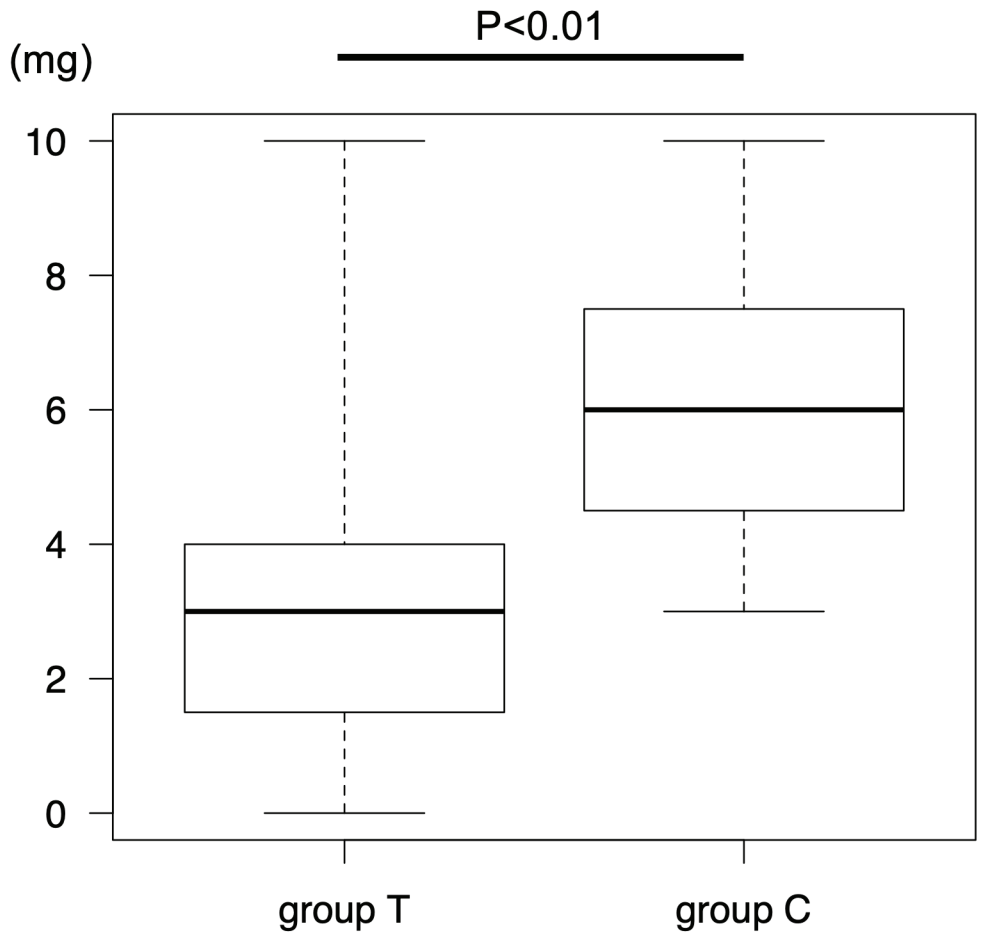

P<0.01
(mg)
10
8
6
4
2
0
group T
group C

Table 1.

Clinical parameter changes of participants in our previous clinical trial

	At initial therapy		At remission		P-value
Serum ferritin, ng/ml	341.9	139.9-589.4	35.9	14.8-56.0	<0.001
Serum KL-6, U/ml	584	496.9-752.1	233	206.5-255.5	<0.001
Anti-MDA5 antibody titer			5	4-8	
FVC, % predicted	88.4	80.2-95.4	104.5	96.7-115.4	<0.001
DLco/VA, % predicted	81.7	73.4-87.8	91.7	76.4-101.3	<0.01
DLco, % predicted	64	56.5-69.4	91.7	76.4-101.3	<0.01

Values are given as the median (interquartile range). Statistical analyses were performed using Wilcoxon signed-rank sum test. Remission data were evaluated at median 2,320 days from initial therapy. FVC, forced vital capacity; DLco/VA, diffusing capacity for carbon monoxide corrected for alveolar volume. We have few data of anti-MDA5 antibody titer at initial therapy because the measurement of anti-MDA5 antibody had not standardized in Japan then.

Table 2.

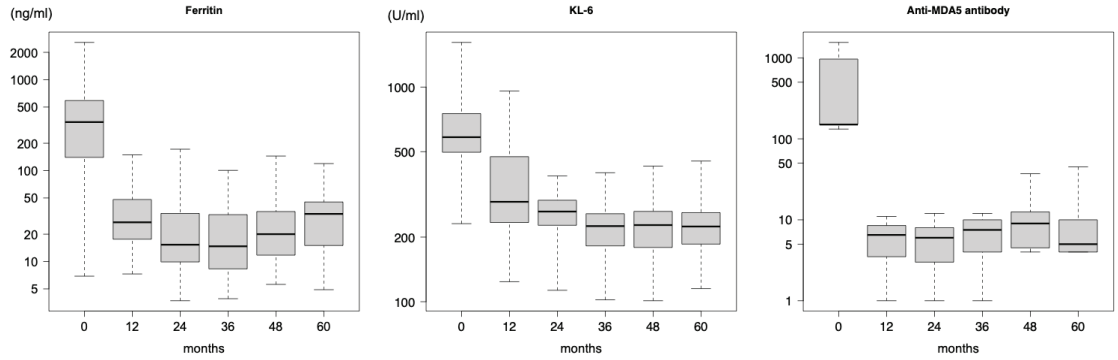
Comparison of clinical characteristics at baseline between group T and C

	Group T (n=56)		Group C (n=12)		P-value
Age, years	48.0	(41.0-58.3)	50.5	(44.8-53.0)	0.89
Female sex, %	73.2		83.3		0.72
Time from onset to treatment, days	69	(46-109)	251	(152-347)	<0.001
SpO_2, %	97	(96-98)	97	(96-97)	0.42
Oxygen therapy, %	4		0		1
LDH, U/L	348.5	(257.5-409.3)	379.0	(292.0-463.0)	0.64
CRP, mg/dL	0.25	(0.1-0.725)	0.20	(0.1-0.50)	0.76
Serum ferritin, ng/ml	357.4	(189.8-658.6)	233.4	(75.3-631.0)	0.36
Serum KL-6, U/ml	588	(497-771)	603	(538-1255)	0.32
FVC, % predicted	85.4	(70.1-100.0)	81.7	(62.2-94.4)	0.60
DLco/VA, % predicted	81.7	(70.0-92.1)	67.8	(58.6-70.7)	<0.01
DLco, % predicted	64.7	(52.5-74.1)	46.1	(35.4-58.0)	<0.01
Treatments					

Only PSL, n	0		3		<0.01
PSL+Tac, n	0		3		<0.01
PSL+CyA, n	0		1		0.18
Only PSL , lately adding Tac, n	0		2		<0.05
Only PSL , lately adding CyA, n	0		2		<0.05
PSL+IVCY, n	0		1		0.18
PSL+Tac+IVCY, n	37		0		<0.001
PSL+CyA+IVCY, n	19		0		<0.05
Initial dose of PSL, mg	55.0	(50.0-60.0)	30	(22.5-45.0)	<0.001
Timing of CNI, days	0	(0-0)	123	(23-255)	<0.001
Timing of IVCY, days	2	(1-5)	34	(34-34)	0.13
Total dose of IVCY, mg	8965	(7888-10590)	0	(0-0)	<0.001
Number of IVCY, count	10	(9-10)	0	(0-0)	<0.001
Rate of using PE, %	18		0		0.19
Follow-up time, days	1895	(1195-2634)	2412	(757-4289)	0.36

3

The values of age at initial therapy, time from onset to treatment, SpO_2, LDH, CRP, serum ferritin, KL-6, FVC predicted, DLco/VA predicted, initial dose of PSL, total dose of IVCY, number of IVCY and follow-up time indicate median (interquartile range) compared with each group using the Wilcoxon test. Other values were assessed by the Fisher's exact test. SpO_2, Oxygen saturation of peripheral artery; LDH, lactate dehydrogenase; CRP, C-reactive protein; KL-6, krebs von den lungen; FVC, forced vital capacity; DLco/VA, diffusing capacity for carbon monoxide corrected for alveolar volume; CNI, calcineurin inhibitor; Tac, tacrolimus; CyA, cyclosporine A; IVCY, intravenous cyclophosphamide; PE, plasma exchange. We described "lately adding Tac/CyA" when these drug added after 3 months of starting PSL.

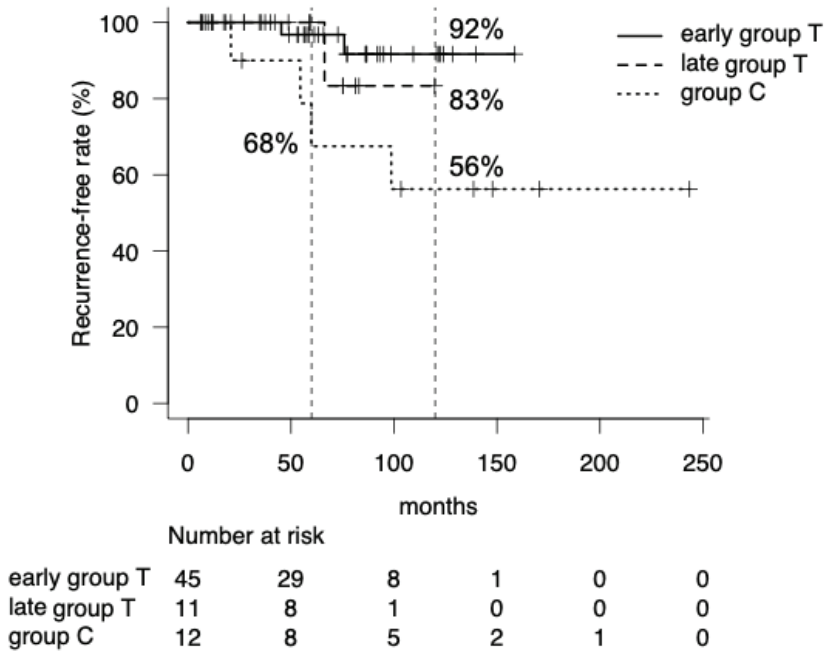


Supplementary Figure S1.

CHANGES IN CLINICAL PARAMETERS OF ANTI-MDA5-POSITIVE DERMATOMYOSITIS WITH INTERSTITIAL LUNG DISEASE

Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median values, and the lines outside the boxes represent the minimum and maximum values.

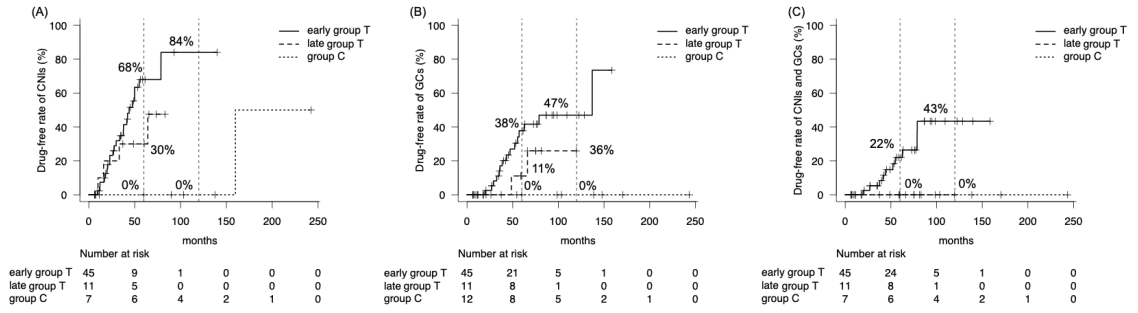
MDA5, melanoma differentiation-associated gene 5; KL-6, Krebs von den Lungen.



Supplementary Figure S2.

RECURRENCE FREE RATES IN EARLY GROUP T, LATE GROUP T AND GROUP C

The recurrence-free rate at 60 months from initial therapy are 97%, 100%, and 68% in early group T, late group T, and group C, respectively. There are not any significant differences among the 3 groups (P=0.064). The cumulative recurrence-free rate was calculated using the Kaplan-Meier method. The log-rank test is also used to compare recurrence free rate.



Supplementary Figure S3.

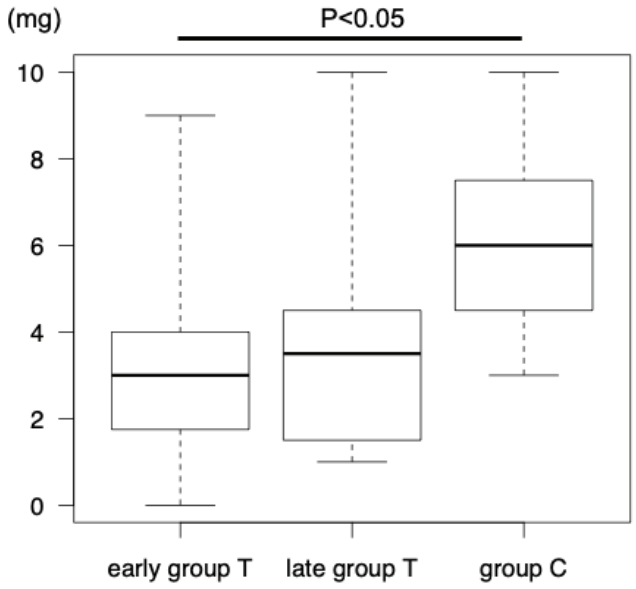
THE CUMULATIVE WITHDRAWAL RATES OF CALCINEURIN INHIBITORS AND/OR GLUCOCORTICOIDS

(A) Overall drug free rate among the early T, late T, and C groups; drug-free rate of CNIs in early group T is significantly higher than in group C ($P<0.01$).

(B) Drug-free rate of GCs in early group T is significantly higher than in group C ($P<0.05$).

(C) Drug-free rates of both CNIs and GCs are not significantly different among the 3 groups. Drug-free rate was calculated using the Kaplan-Meier method and the log-rank test with Bonferroni adjustment.

CNIs, calcineurin inhibitors; GCs, glucocorticoids.



Supplementary Figure S4.

THE MAINTENANCE DOSE OF PREDNISOLONE AT 36 MONTHS FROM INITIAL THERAPY

Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median values, and the lines outside the boxes represent the minimum and maximum. The median doses of prednisolone are 3.0 mg/day, 3.5 mg/day, and 6.0 mg/day in the early group T (n=35), late group T (n=10), and group C (n=8), respectively. The median dose in the early group T is significantly lower than that in group C, estimated by using Wilcoxon test with Bonferroni adjustment (P=0.011).

Supplementary table S1.

Comparison of baseline clinical characteristics between the early T, late T, and C groups

	Early group T (45)		Late group T (11)		Group C (12)		P-value
Age, years	48	(41-59)	48	(41-57)	51	(45-53)	0.90
Female sex, %	71		82		83		0.71
Time from onset to treatment, days	68	(44-109)	82	(51-106)	251	(152-347)	<0.001
SpO_2, %	97	(96-98)	97	(95-98)	97	(96-97)	0.65
Oxygen therapy, %	2		9		0		0.33
LDH	350	(272-414.5)	328	(224-371)	379	(292-463)	0.46
CRP	0.30	(0.10-0.75)	0.22	(0.10-0.34)	0.20	(0.10-0.50)	0.78
Serum ferritin, ng/ml	357.4	(188.3-647.7)	417.3	(199.3-829.1)	233.4	(75.3-631.0)	0.65
Serum KL-6, U/ml	627.0	(505.7-842.0)	542.5	(398.5-644)	603.0	(538.0-1255)	0.20
FVC, % predicted	84.8	(71.5-95.8)	85.5	(73.2-94.6)	81.7	(62.2-94.4)	0.85
DLco, % predicted	63.7	(49.5-72.5)	77.5	(63.3-86.8)	46.1	(35.4-58.0)	<0.01
Treatments							
Only PSL, n	0		0		3		<0.01
PSL+Tac, n	0		0		3		<0.01
PSL+CyA, n	0		0		1		0.34
Only PSL, late addition of Tac, n	0		0		2		0.053
Only PSL, late addition of CyA, n	0		0		2		0.053
PSL+IVCY, n	0		0		1		0.34
PSL+Tac+IVCY, n	28		9		0		<0.001
PSL+CyA+IVCY, n	17		2		0		<0.05
Initial dose of PSL, mg	55.0	(50.0-60.0)	50.0	(42.5-50.0)	30.0	(22.5-45.0)	<0.001
Timing of CNI	0	(0-0)	10	(5-22)	123	(23-255)	<0.001
Timing of IVCY	1	(1-3)	22	(16-36)	34	(34-34)	<0.001
Total dose of IVCY, mg	9300	(8000-10800)	8550	(6690-8925)	0	(0-0)	<0.001
Number of IVCY, count	10	(9-10)	10	(8-10)	0	(0-0)	<0.001
Rate of using PE, %	20		9		0		0.20
Follow-up time, days	1867	(1085-2797)	2017	(1636-2384)	2413	(757-4289)	0.66

The values of age at initial therapy, time from onset to treatment, SpO_2, LDH, CRP, serum ferritin, KL-6, FVC predicted, DLco/VA predicted, initial dose of PSL, total dose of IVCY, number of IVCY, and follow-up time indicate median (interquartile range) compared between each group using the Wilcoxon test. Other values were assessed by the Fisher's exact test. SpO_2, Oxygen saturation of peripheral artery; LDH, lactate dehydrogenase; CRP, C-reactive protein; KL-6, krebs von den lungen; FVC, forced vital capacity; DLco/VA, diffusing capacity for carbon monoxide corrected for alveolar volume; CNI, calcineurin inhibitor; Tac, tacrolimus; CyA, cyclosporine A; IVCY, intravenous cyclophosphamide; PE, plasma exchange. We described "late addition of Tac/CyA" when these drugs were added after 3 months of starting PSL.

Supplementary Table S2.

Comparison of clinical characteristics at remission between group T and C

	Group T (n=41)		Group C (n=5)		P-value
Time from initial therapy, days	2289	(1721-2847)	2338	(2329-2341)	0.407
Serum ferritin, ng/ml	39.0	(19.3-63.0)	26.8[a]	(26.8-30.6)	0.479
Serum KL-6, U/ml	224.5	(174.8-268.3)	508.0	(306.0-635.0)	<0.05
Anti-MDA5 antibody titer	4	(4-8)	14[b]	(14-14)	0.118
FVC, % predicted	106.2	(95.6-113.6)	88.8[C]	(87.2-90.5)	0.0776
DLco, % predicted	79.2	(69.6-83.3)	67.3[C]	(64.0-70.6)	0.279

The values of time from initial therapy, serum ferritin, KL-6, Anti-MDA5 antibody titer, FVC predicted, and DLco/VA predicted indicate median (interquartile range) compared between each group using the Wilcoxon test. Some patients in group C are not fully tested for remission, [a]n=3, [b]n=1, [c]n=2. FVC, forced vital capacity; DLco/VA, diffusing capacity for carbon monoxide corrected for alveolar volume.

Supplementary Table S3.

Detailed clinical course of patients who had lung relapse

Group	Age (years)	Sex	Relapse time (months)	Therapy just before relapse	Re-induction therapy	Outcome after re-induction therapy
Early group T	41	F	45	PSL: 6mg	GCs (M) and IVCY (2)	Dead by breast cancer after 9 months
	58	F	75	PSL: 10mg+Tac: 3mg	GCs (M), CNIs, IVCY (6) and PE	Maintaining complete remission for 21 months
Late group T	43	F	66	PSL: 2mg	GCs (H), CNIs and IVCY (4)	Maintaining complete remission for 85 months
Group C	45	M	98	PSL: 0.5mg	GCs (M), CNIs and IVCY (6)	Maintaining complete remission for 30 months
	47	F	59	PSL: 5mg+Tac: 2mg	GCs (L), CNIs and IVCY (6)	Relapse of skin and lung lesions after 23 months
	50	F	20	PSL: 4mg	GCs (M) and IVCY (6)	Relapse of skin lesions after 10 months
	56	F	54	PSL: 1.5mg	GCs (M), CNIs and IVCY (6)	Dead by suicide after 4 months

PSL, prednisolone; Tac, tacrolimus; GCs (H), high-dose glucocorticoid; GCs (M), moderate-dose glucocorticoid; GCs (L), low-dose glucocorticoid; CNI, calcineurin inhibitor; IVCY, intravenous cyclophosphamide; PE, plasma exchange. The number in the parenthesis following “IVCY” represents the number of IVCY.

Supplementary Table S4.

Serious adverse events between group T and C

	Group T (56)		Group C (12)	
Number of patients	10	(17.9)	3	(25.0)
Number of serious adverse events	14		6	
Pneumonia	2	(3.6)	0	(0.0)
Pneumocystis pneumonia	1	(1.8)	0	(0.0)
Lung abscess	1	(1.8)	0	(0.0)
Enteritis	1	(1.8)	3	(25.0)
Herpes zoster	2	(3.6)	1	(8.3)
Cellulitis	0	(0.0)	2	(16.7)
Cytomegalovirus retinitis	1	(1.8)	0	(0.0)
Pyelonephritis	1	(1.8)	0	(0.0)
Sinusitis	1	(1.8)	0	(0.0)
Appendicitis	1	(1.8)	0	(0.0)
Reversible posterior leukoencephalopathy syndrome	1	(1.8)	0	(0.0)
Cerebral infarction	1	(1.8)	0	(0.0)
Pulmonary alveolar proteinosis	1	(1.8)	0	(0.0)
Malignancy thought to be induced by cyclophosphamide	0	(0.0)	0	(0.0)

Serious adverse events are defined as unexpected admissions, such as infection or emergency surgery. Multiple occurrences in one patient are counted. Data are n (%).

Supplementary Table S5.

Serious adverse events among early group T, late group T, and group C

	Early group T (45)		Late group T (11)		Group C (12)	
Number of patients	8	(17.8)	2	(18.2)	3	(25.0)
Number of serious adverse events	10		4		6	
Pneumonia	2	(4.4)	0	(0.0)	0	(0.0)
Pneumocystis pneumonia	1	(2.2)	0	(0.0)	0	(0.0)
Lung abscess	1	(2.2)	0	(0.0)	0	(0.0)
Enteritis	0	(0.0)	1	(9.1)	3	(25.0)
Herpes zoster	1	(2.2)	1	(9.1)	1	(8.3)
Cellulitis	0	(0.0)	0	(0.0)	2	(16.7)
Cytomegalovirus retinitis	1	(2.2)	0	(0.0)	0	(0.0)
Pyelonephritis	1	(2.2)	0	(0.0)	0	(0.0)
Sinusitis	0	(0.0)	1	(9.1)	0	(0.0)
Appendicitis	1	(2.2)	0	(0.0)	0	(0.0)
Reversible posterior leukoencephalopathy syndrome	0	(0.0)	1	(9.1)	0	(0.0)
Cerebral infarction	1	(2.2)	0	(0.0)	0	(0.0)
Pulmonary alveolar proteinosis	1	(2.2)	0	(0.0)	0	(0.0)
Malignancy thought to be induced by cyclophosphamide	0	(0.0)	0	(0.0)	0	(0.0)

Serious adverse events are defined as unexpected admissions, such as infection or emergency surgery. Multiple occurrences in one patient are counted. Data are n (%).