Complements

Contents

Inclusion and exclusion criteria

1. Inclusion criteria

1. Male or female, aged 18-70 years (including 18 and 70 years) at the time of screening visit.

2. The diagnosis conforms to the 1975 Bohan & Peter recommended criteria[13, 14] and has been diagnosed for more than 3 months before entering the group for screening

3. Glucocorticoid (≤1mg/kg/d prednisone or other hormones with equivalent dose), DMARDs (such as methotrexate, hydroxychloroquine, azathioprine, mycophenolate mofetil, leflunomide, cyclosporine, etc.) must be treated stably for 12 weeks, without increasing hormone dose or other immunosuppressants in the whole study. If the registered doctor plans to stop using the current immunosuppressants or glucocorticoids, it is necessary to follow the elution period before ehrollment. Each drug shall meet the following elution periods.

- Glucocorticoid -4 weeks
- Immunosuppressants (including methotrexate, azathioprine, cyclosporine, tacrolimus, leflunomide, mycophenolate mofetil)-12 weeks
- IVIg or cyclophosphamide-12 weeks
- Rituximab -6 months
- Other biological agents (infliximab, adalimumab, etanercept, anakinra, etc.) -12 weeks.

4. When participating in the trial, the patient must be given a written notice of consent, and the patient is expected to comply with the requirements of the study follow-up plan and other protocols.

5. Active disease activity: cutaneous VAS score on the Myositis Disease Activity Assessment Tool (MDAAT)≥3cm and core-set measures(CSM) at least 3 abnormalities (dermatomyositis), or MMT-8≤125/150 and CSM at least 2 abnormalities:

1) Patients global assessment, the minimum value of 10 cm visual analogue scale (VAS) is 2.0 cm.
2) Physicians global assessment, the minimum value on the 10 cm VAS scale is 2.0 cm.
3) Health Assessment Questionnaire (HAQ) disability index, the minimum value is 0.25
4) The increase of at least one muscle enzyme [including creatine kinase (CK), aldolase, lactate dehydrogenase (LDH), alanine aminotransferase (ALT) or aspartate aminotransferase (AST)], the lowest level is 1.3 × the upper limit.
5) Global extramuscular disease activity score, with a minimum value of 1.0 cm on the 10 cm VAS scale. This measure is a comprehensive evaluation of doctors, based on the evaluation of physical fitness, skin, bone, gastrointestinal, lung and heart scale activity scores in MDAAT.

2. Exclusion criteria

Any subject meeting any of the following criteria should be excluded:

1. Use of rituximab or other monoclonal antibodies within 6 months.

2. Treated with high-dose glucocorticoid (> 1 mg/kg/d) within one month.

3. Serious complications: including heart failure (≥ NYHA III), renal insufficiency (creatinine clearance rate ≤30 ml/min), liver insufficiency (serum ALT or AST is higher than the normal upper limit by more than 3 times, or total bilirubin is more than the normal upper limit)

4. Other serious, progressive or uncontrollable hematological, gastrointestinal, endocrine, pulmonary, cardiac, neurological or brain diseases (including demyelinating diseases, such as multiple sclerosis).

5. Allergy, high reactivity or intolerance of IL-2 or its excipient.

6. Suffering from serious infection (including but not limited to hepatitis, pneumonia, bacteremia, pyelonephritis, Epstein-Barr virus and tuberculosis infection), or being hospitalized due to infection, or using intravenous antibiotics to treat infection 2 months before the first dose of IL-2.

7. In the first 3 months before using IL-2, the chest image showed the abnormal malignant tumor or current active infection (including tuberculosis).

8. Infected with HIV (seropositive for HIV antibody) or hepatitis C (seropositive for Hep C antibody). If the serum is positive, it is recommended to consult a doctor with expertise in the treatment of HIV or hepatitis C virus infection.

10. Any known malignant tumor or history of malignant tumor in the past 5 years (except for non-melanoma skin cancer, which showed no signs of recurrence or cervical tumor cured by surgery within 3 months before enrollment).

11. Uncontrolled mental or emotional disorders, including a history of drug and alcohol abuse in the past three years, which may hinder the successful completion of the research.

12. Accept or expect to receive any live virus or bacterial vaccine within 3 months before the first injection or within 4 months after the last injection of IL-2. BCG was inoculated within 12 months after screening.

13. Pregnant and lactating women are reluctant to use medically approved contraceptive measures during treatment and within 12 months after treatment.

14. Men whose partners have fertility potential but are unwilling to use appropriate medically approved contraceptive measures during treatment and within 12 months after treatment.

15. Adolescent DM or PM.

Table S1. Antibodies used in flow cytometric analysis.

Antibody	Parameter	Item No.	Reactivity	Validation
CD3	APC-H7	641397	human	BD
CD4	FITC	340133	human	BD
CD8	PERCP	341051	human	BD
CD25	PE	341009	human	BD
CD197 (CCR7)	BV421	562555	human	BD
PD-1	PE-CY7	561272	human	BD
CD185 (CXCR5)	AF647	558113	human	BD
CD3	PERCP	652831	human	BD
CD19	PE-cy7	341113	human	BD
CD56	APC	341025	human	BD
CD16	PE	347617	human	BD
Foxp3	AF647	560045	human	BD

Table S2. Baseline characteristics of patients with IIMs (n=18).

Characteristic	Value
Age, year, median (range)	50 (42-61)
Female/Male	20/3
Diagnosis	
DM, n (%)	10 (55.56)
ASS, n (%)	6 (33.33)
IMNM, n (%)	2 (11.11)
Duration of IIMs, year, median(range)	6 (2-8)
Prednisone dose, mg/day, median (range)	15.5±11.5
Use of concomitant agents, n (%)	
Hydroxychloroquine	3 (16.67)
Cyclophosphamide	4 (22.22)
Cyclosporine	5 (27.78)
Mycophenolatemofetil	2 (11.11)
Azathioprine	1 (5.56)
Autoantibodies, n (%)	
Anti-synthetase antibody	6 (33.33)
Anti-Mi-2α	1 (5.56)
Anti-Mi-2β	0 (0)
Anti-TIF-1γ	0 (0)
Anti-MDA5	2 (11.11)
Anti-NXP2	0 (0)
Anti-SAE1	0 (0)
Anti-Ku	1 (5.56)
Anti-PM-Scl100	0 (0)
Anti-PM-Scl75	2 (11.11)
Anti-SRP	3 (16.67)
Anti-Ro-52	8 (44.44)

Data are presented as median (IQR) or n (%). IIMs, idiopathic inflammatory myopathies. DM, dermatomyositis. ASS, antisynthetase syndrome. IMNM, immune-mediated necrotizing myopathy. MDA5, melanoma differentiation-associated gene 5.

Table S3. Details of baseline characteristics of patients with IIMs (n=18).

Patient number	Age (years)	Duration (years)	Previous treatments	Concomitant treatments (years)	Prednisone dose (mg per day)	Clinical and serological manifestations
1	64	6	CTX	HCQ-5years, MMF-3 years	5	Mechanic's hand, arthritis, ILD, anti-EJ antibodies, anti-Ro-52 antibodies, ESR 48 mm/h,
2	54	10	-	CTX-6 months CSA-6 months	15	Myasthenia, ILD, creatine kinase 3822 U/L, ESR 32 mm/h, anti-SRP antibodies,
3	61	28	HCQ, Iguratimod	CTX-6months	15	ILD, creatine kinase 369 U/L, ESR 23 mm/h, anti-Ku antibodies, anti-PM-Scl-100 antibodies
4	70	6	HCQ	-	7.5	Mechanic's hand, Heliotrope rash
5	43	3	CTX, HCQ	MMF-18 months, CSA-3 months	30	Fatigue, Heliotrope rash, diffuse pigmentation, arthritis, ILD, anti-Mi-2α antibodies, anti-PL-7 antibodies, anti-Ro-52 antibodies
6	59	7	CTX HCQ	CSA-3 months	15	Fatigue, Heliotrope rash, Gottron's sign, perlungual erythematosus, myasthenia, ILD, creatine kinase 518 U/L,
7	22	13	MTX	CSA-4 months, HCQ-12 months, thalidomide-12 months	0	Fatigue, Heliotrope rash, pigmentation, anti-PM-Scl-75 antibodies
8	50	6	CTX CSA	AZA-30 months, HCQ-12 months	5	Rash, ILD, anti-Ro-52 antibodies
9	42	2	CTX, MTX, thalidomide	CSA-3 months	10	Subcutaneous calcification
10	28	2	AZA, MMF, Secukinumab	CSA-8 months	15	Myasthenia, creatine kinase 5427 U/L, anti-HMGCR antibodies
11	68	7	MTX	HCQ-4 years	5	Gottron's sign, myasthenia, creatine kinase 483 U/L, anti-PM-Scl-75 antibodies, anti-Ro-52 antibodies
12	65	1	-	CTX-3 months	37.5	Rash, ILD, creatine kinase 218 U/L, anti-PL-7 antibodies, anti-Ro-52 antibodies
13	43	1	HCQ	CSA-7 months	12.5	Mechanic's hand, Heliotrope rash, Gottron's sign, V sign, perlungual erythematosus, arthritis, ILD, anti-MDA5 antibodies, anti-PL-12 antibodies, anti-Ro-52 antibodies
14	45	0.3	-	-	0	Gottron's sign, ILD
15	63	20	MTX, MMF	CTX-7 months	12	Heliotrope rash, Gottron's sign, perlungual erythematosus, myasthenia,
16	26	2	-	CSA-18 months	12.5	Heliotrope rash, Gottron's sign, V sign, perlungual erythematosus, ILD, creatine kinase 220 U/L, anti-MDA5 antibodies,
17	27	5	MMF, AZA	CSA-8 months	15	Mechanic's hand, Gottron's sign, arthritis, ILD, anti-Jo-1 antibodies, anti-Ro-52 antibodies
18	54	8	CTX	MMF-28 months	5	Heliotrope rash, Gottron's sign, perlungual erythematosus, ILD, creatine kinase 292 U/L, ESR 35, CRP 25.4, anti-PL-12 antibodies, anti-Ro-52 antibodies

CTX, cyclophosphamide. HCQ, hydroxychloroquine. MTX, methotrexate. CSA, ciclosporin. MMF, mycophenolate mofetil. AZA, azathioprine. ILD, interstitial lung disease. MDA5, melanoma differentiation-associated protein 5.

Table S4. Clinical responses to low-dose IL-2 treatment in patients with IIMs.

Patient Number	Clinical Response description	Description of IL-2 related adverse effects
1	1. Remission in Mechanic's hand and arthritis in 12 weeks while relapse at week 24. 2. Improvement of total HRCT Score from 455 to 440. 3. ESR was decreased from 48 mm/h at week 0 to 29 mm/h at week 12 and 33 mm/h at week 24 mm/h.	Local reactions at injection sites.
2	1. She could get into the car without the help from other people. 2. There was no obvious improvement of muscle weakness, enzyme or ILD.	None
3	1. Creatine kinase decreased from 369U/L to 70U/L. 2. Improvement of total HRCT Score from 455 to 440. 3. ESR was decreased from 23 mm/h at week 0 to 13 mm/h at week 24.	None
4	1. Remission in Mechanic's hand and Heliotrope rash.	Local reactions at injection sites.
5	1. Improvement of fatigue, Heliotrope rash and arthritis. . 2. There was no obvious improvement in pigmentation all over the body.	Fever
6	1. Remission in rash all over the body. 2. Remission in fatigue and muscle weakness. 3. Creatine kinase was decreased from 518 U/L to 220 U/L.	None
7	1. Remission in pigmentation, fatigue and Heliotrope rash.	None
8	1. Remission in rash.	None
9	1. Subcutaneous calcification of left lumbar was shrunk with scab.	None
10	1. Creatine kinase decreased from 5427U/L to 5136U/L 2. There was no obvious improvement of muscle weakness.	None
11	1. Increase of MMT-8 (0-150) from 114 to 130. 2. Creatine kinase decreased from 483U/L to 292U/L 3. Remission in Gottron's sign.	Local reactions at injection sites.
12	1. Remission in rash. 2. Creatine kinase decreased from 218 U/L to 51 U/L.	None
13	1. Remission in perlungual erythematosus. 2. Slight improvement in Mechanic's hand, Heliotrope rash, Gottron's sign and V sign.	None
14	1. The Gottron's sign was improved slightly.	Local reactions at injection sites.
15	1. Slight improvement in Gottron's sign, Heliotrope rash and perlungual erythematosus. 2. There was no obvious improvement of myasthenia.	Chills
16	1. Slight improvement in periorbital and facial rash. Heliotrope rash, Gottron's sign, V sign and perlungual erythematosus. 2. Creatine kinase decreased from 220 U/L to 120 U/L.	None
17	1. No obvious improvement of Gottron's sign, Mechanic's hands, arthritis or ILD.	None
18	1. Slightly improvement of Heliotrope rash. 2. Remission in Gottron's sign.	None

ESR, erythrocyte sedimentation rate. IL-2, inerleukin-2. ILD, interstitial lung disease.

Table S5. Response of ILD to low-dose IL-2 treatment (n=10).

Characteristics	baseline	Week 24	P value
%VC (% of predicated)	75.03±18.2	86.63±23.33	0.456
FEV1/FVC (%)	93.12±8.54	54±44.88	0.474
DLco (ml/min/mmHg)	6.07±2.58	4.54±1.03	0.680
DLco (% of predicated)	66.29±20.84	58.03±11.2	0.708
HRCT			
OP, n (%)	4 (40)	4 (40)	1.000
NSIP, n (%)	7 (70)	7 (70)	1.000
OP+NSIP, n (%)	4 (40)	4 (40)	1.000

%VC, percent vital capacity; FEV1/FVC, forced expiratory volume in 1 second/forced vital capacity; DLco, diffusing capacity for carbon monoxide; HRCT, high-resolution computed tomography; OP, organizing pneumonia; NSIP, non-specific interstitial pneumonia.

Table S6. Adverse events during low-dose IL-2 administration (n=18)

Adverse events	Number	%
Injection-site reaction	5	27.78
Fatigue	0	0
Fever	2	11.11
Chills	1	1.96
Nausea	1	1.96
Infection	0	0

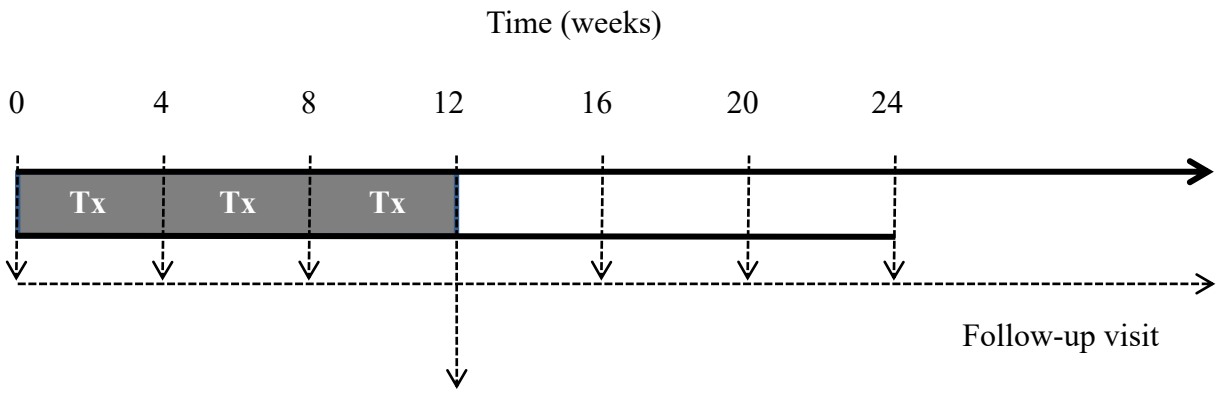
Figure legends.

Figure S1. Study design.

Figure S2. Change of percentage and absolute number of Th17 in peripheral blood of IIMs patients after the treatment of low-dose IL-2. IIMs, idiopathic inflammatory myopathies.

Figure S3. Difference of Treg subset distribution and IL-2 signaling in IIMs with HC. (A) Flow cytometry analysis of Treg subsets based on FOXP3 and CD45RA expression patterns. (B) Treg subsets based on FOXP3 and CD45RA expression in IIMs and HC. (C) Quantification of CD25 expression on Treg subsets in IIMs and HC. IIMs, idiopathic inflammatory myopathies. HC, healthy controls.

Figure S4. Change of Treg subsets and expression of CD25 on Treg subsets in peripheral blood of IIMs patients after the treatment of low-dose IL-2. (A) Change of Treg subsets based on FOXP3 and CD45RA expression after low-dose IL-2 therapy. (B) Change of quantification of CD25 expression on Treg subsets after IL-2 therapy. IIMs, idiopathic inflammatory myopathies. HC, healthy controls.



Tx 1 million units of IL-2 subcutaneously every other day for 3 months

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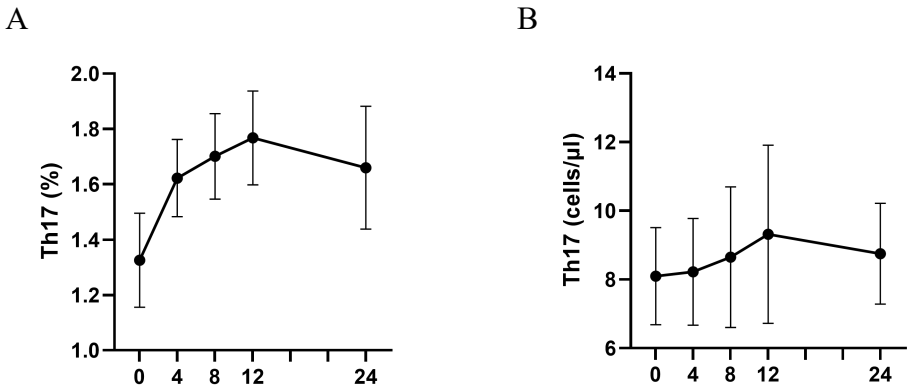

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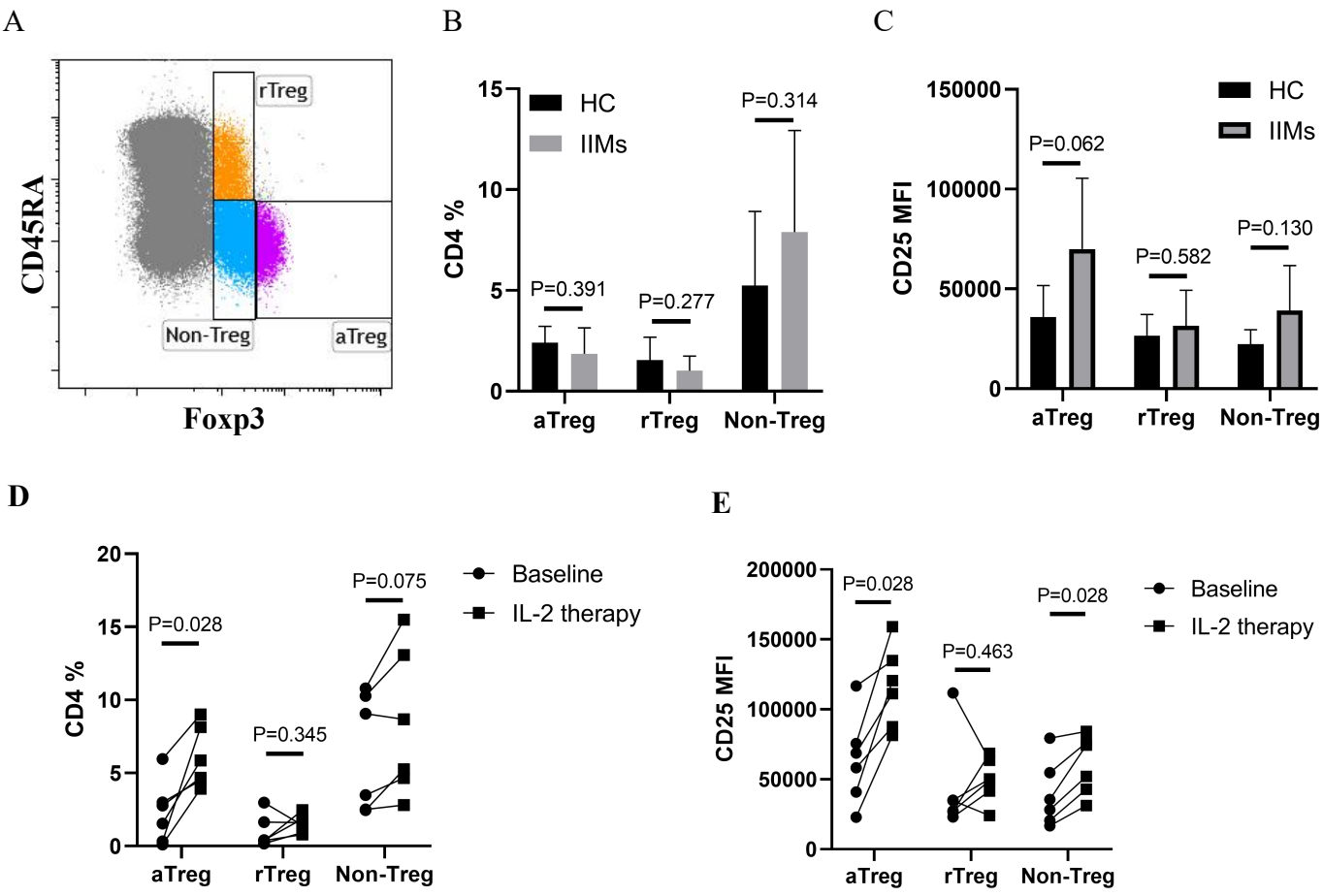

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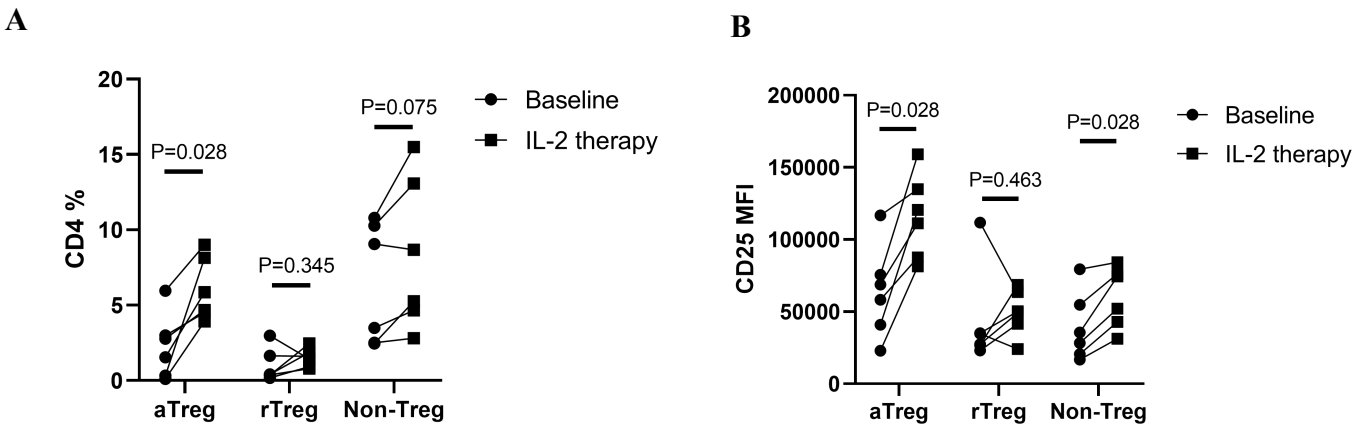

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