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Predictive biomarkers for low-dose IL-2 therapy efficacy in systemic lupus erythematosus: a clinical analysis

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Abstract

Background Low-dose IL-2 (Ld-IL2) has shown favorable therapeutic effects in systemic lupus erythematosus (SLE) therapy. However, previous clinical trials reported an SLE Responder Index-4 (SRI-4) response rate of 65.52%-68%, with approximately half failing to achieve the primary endpoint by week 24. Our study aims to determine the real-world use of Ld-IL2 and to identify determinants of its effectiveness in SLE.

Methods We pooled data from 342 SLE patients undergoing sequential Ld-IL2 treatment, with 314 persisting for over 3 months were included in effectiveness and prediction analyses. All patients were categorized into responder ($n=136$) and non-responder group ($n=178$) according to SRI-4. Lupus Low Disease Activity State (LLDAS) was also analyzed to validate our results.

Results Rash, lower complement 3 (C3), and renal involvement including urine protein, urine occult blood and urine casts emerged as prominent predictors of achieving SRI-4. Adjusting for baseline values using the ratio of change to baseline revealed significant differences in CD4+T cell immune profiles between responders and non-responders. ROC analysis confirmed a satisfactory performance of rash, renal involvement, percentage change of CD4+T cells, and C3 in predicting SRI-4, yielding an AUC of 0.933. LLDAS analysis showed that hematological involvements and lower CLA+Treg were potent predictive markers in LLDAS attainment. Conversely, renal involvement failed to have significant association in achieving LLDAS. The analysis of background therapy in SLE patients showed that MMF was more likely to reach the SRI-4 response with the combination of Ld-IL2.

Conclusions These findings uncovered the predictors of Ld-IL2 treatment efficacy in SLE patients and provided guidance to physicians for rational utilization.

Keywords Low-dose IL-2, Systemic lupus erythematosus, SLE Responder Index-4 (SRI-4), Efficacy responses, Predictive factors

Background

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by immune-system aberrations and the production of autoantibodies and immune complexes, resulting in the inflammation across multiple organs [1]. Given the advances in therapeutic strategies for SLE over the past decades, the satisfactory efficacy of low-dose IL-2 (Ld-IL2) has sparked excitement for the therapeutic exploration in the context of SLE [2, 3]. It provides a means to mitigate side

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effects commonly associated with conventional therapy such as infection, myelosuppression, and gastrointestinal reaction [4]. Thus, it is vital for physicians and patients with SLE to accurately predict the likelihood of achieving SLE Responder Index-4 (SRI-4) at the initiation of Ld-IL2 therapy, which allows for the optimal decision-making of treatment goals and strategies.

Interleukin-2 (IL2), a crucial cytokine in immune regulation, plays a pivotal role in the activation and proliferation of T cells, as well as the development of regulatory T cells (Tregs) [5]. The rationale behind Ld-IL2 application in SLE attributes to its capacity to enhance Treg function, which is often impaired in autoimmune diseases [6]. Previous studies have demonstrated the safety and efficacy of Ld-IL2 in small cohorts or clinical trials, but they reported an SRI-4 response rate of 65.52%-68%, with approximately half of patients not achieving the primary endpoint by week 24 [2, 7, 8]. Consequently, detailed and comprehensive evaluations of Ld-IL2 use in real-world settings are scarce and there are no robust predictive markers for SLE patients to achieve a stable disease remission following initial Ld-IL2 treatment. To address this gap, we assembled data from 369 SLE subjects prescribed with Ld-IL2 and validated the predictors for achieving SRI-4.

Methods

Study patients

A total of 369 SLE patients who were prescribed with Ld-IL2 were initially enrolled from Peking university people's hospital between October 2018 and May 2024. Eligible patients were aged 18 years or older evaluated by their physicians and fulfilled the 1997 revised classification criteria of the American College of Rheumatology [9]. Excluding patients who refused to use Ld-IL2 ($n=12$) and those lost to follow-up ($n=15$), we collected data from 342 SLE patients with continuous Ld-IL2 treatments. The effectiveness and prediction analyses included 314 individuals with over three cycles treatment (Fig. 1). The study was approved by the ethical committee of Peking University People's Hospital (approval number 2022PHB013-001) and all patients provided written consent.

Definitions and data collection

Ld-IL2 treatment course consisted of a dose of 1 million IU subcutaneously every other day for 2 weeks, followed by a 2-week break as one treatment cycle [2, 10, 11]. Patients were classified into two groups: responders were defined as meeting all of the following criteria: a $\geq$4-point reduction from baseline in SLE Disease Activity Index (SLEDAI)-2 K score, no new disease activity

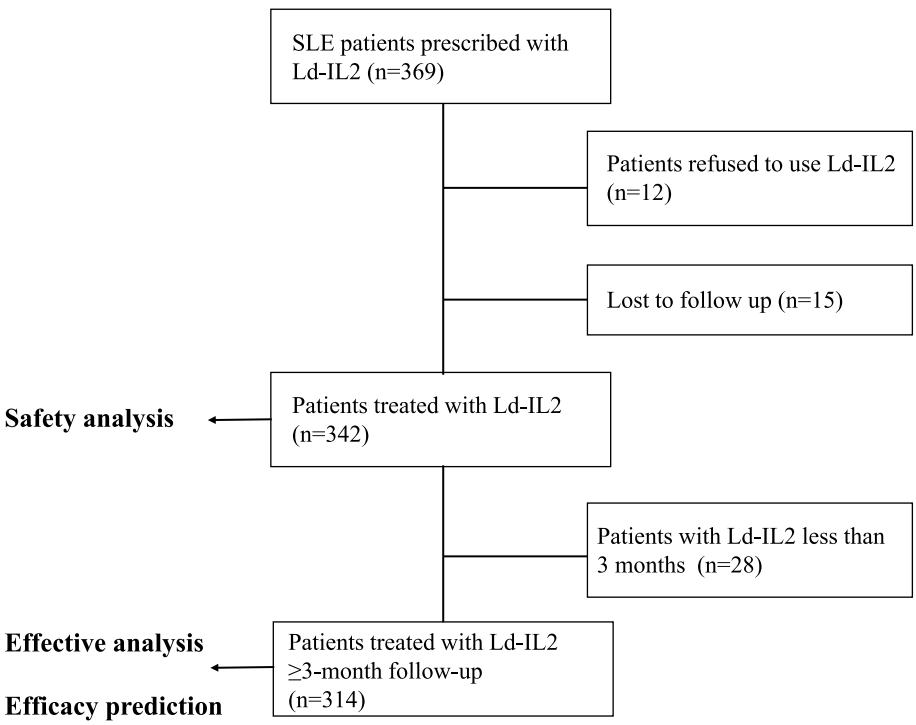

Fig. 1 Flow chart of data processing and exclusions. After excluding patients who refused to use Ld-IL2 ($n=12$) and lost to follow-up ($n=15$), 342 patients were enrolled for safety analysis. Only 314 patients with a minimum of 3 months of persistence were included for effective analysis and efficacy prediction. Ld-IL2: low-dose IL-2

measured by a new British Isles Lupus Assessment Group (BILAG) grade A or > 1 BILAG grade B score, and no worsening (an increase of < 0.3 points from baseline) in the physician global assessment (PGA) of disease activity [12]. Thus, non-responders included patients who achieved less than 4 points reduction in SLEDAI from baseline, followed by an increase in PGA score and one or more organ domains by BILAG index. Simultaneously, lupus low disease activity state (LLDAS) was defined a SLEDAI score of 4 or less, no new disease activity, a PGA score of 1 or less, and a prednisone dose of 7.5 mg/day or less. Any adjustment in the patient's treatment regimen

Table 1 Demographic and baseline characteristics of patients

Characteristics	All patients (*n* = 342)	Responders (*n* = 136)	Non-responders (*n* = 178)	*P* value
Age (years), mean (SD)	39.0 (15.0)	38.6 (14.7)	39.8 (14.8)	0.391
Female, n (%)	312 (91.2)	123 (91.1)	156 (87.6)	0.559
Disease duration (years), median (range)	8 (5–14)	9 (5–16)	7 (5–13)	0.316
SELENA-SLEDAI, median (range)	8 (5–12)	11 (8–16)	9 (6–14)	0.626
Overall BILAG-2004 A/B grades				
≥ 1 A grade	32 (9.4)	16 (11.9)	15 (8.4)	0.424
No A grade or ≥ 2 B grades	25 (7.3)	10 (7.4)	15 (8.4)	0.843
PGA score, mean ± SD				
Symptom at baseline, no. (%)				
Rash, n (%)	91 (26.6)	43 (31.9)	39 (22.4)	0.643
Oral ulcers, n (%)	23 (6.7)	9 (6.7)	11(6.1)	0.606
Serositis, n (%)	16 (4.7)	6 (4.4)	8 (4.5)	0.563
Alopecia, n (%)	105 (30.7)	48 (35.6)	52 (29.1)	0.584
Arthritis, n (%)	110 (31.9)	54 (40.0)	55 (30.7)	0.722
Leukopenia, n (%)	65 (18.8)	25 (18.5)	33 (18.5)	0.978
Thrombocytopenia, n (%)	68 (19.7)	31 (23.0)	32 (18.1)	0.288
Renal involvement, n (%)	72 (20.9)	40 (31.1)	30 (16.8)	0.571
Neurological involvement, n (%)	12 (3.5)	4 (3.0)	7 (3.9)	0.824
Clinical parameters				
WBC, × 109/L, median (range)	5.80 (4.24–8.09)	6.13 (4.38–8.35)	5.50 (4.20–7.82)	0.267
Lymphocyte, × 109/L, median (range)	23.65 (15.35–30.20)	22.20 (14.40–29.95)	24.60 (17.05–30.25)	0.083
Neutrophil, × 109/L, median (range)	65.45 (56.85–75.65)	68.80 (59.15–77.05)	64.20 (55.98, 73.25)	0.030
Platelet, × 1012/L, median (range)	199 (147–260)	197 (133, 251)	199 (153, 265)	0.430
Hemoglobin, median (range)	122 (111–134)	120 (107, 131)	124 (112, 136)	0.030
IgA, g/L, median (range)	2.35 (1.46–3.09)	2.23 (1.37, 2.87)	2.52 (1.68, 3.19)	0.081
IgG, g/L, median (range)	12.65 (8.76–16.31)	11.60 (8.20, 15.00)	13.25 (9.60, 16.95)	0.050
IgM, g/L, median (range)	0.87 (0.54–1.26)	0.87 (0.52, 1.33)	0.86 (0.57, 1.26)	0.973
C3, g/L, median (range)	0.76 (0.60–0.93)	0.71 (0.57, 0.91)	0.81 (0.63, 0.96)	0.017
C4, g/L, median (range)	0.17 (0.12–0.22)	0.16 (0.12, 0.21)	0.17 (0.12, 0.22)	0.284
Anti-dsDNA, IU/mL, median (range)	25.15 (9.28–56.28)	28.80 (10.15, 56.00)	18.00 (8.50–56.80)	0.463
AnuA, IU/mL, median (range)	12.07 (2.69–46.58)	16.5 (5.04, 73.66)	9.67 (1.20, 28.71)	0.051
Urine protein, g/24 h, median (range)	0.53 (0.18–1.77)	0.68 (0.24, 2.20)	0.26 (0.14–1.34)	0.009
Treatments at baseline, n (%)				
GC, mg/d, median (range)	15.0 (7.5–30.0)	15.0 (10.0, 42.5)	12.0 (7.5, 30.0)	0.031
Hydroxychloroquine	282 (82.5)	103 (76.3)	133(74.3)	0.754
Cyclosporine	34 (10.3)	20 (14.8)	27 (15.1)	0.888
Mycophenolate mofetil	84 (25.4)	70 (51.9)	69 (38.5)	0.046
Leflunomide	15 (4.5)	7 (5.2)	13 (7.3)	0.580
Cyclophosphamide	17 (5.1)	15 (11.1)	16 (8.9)	0.516
Azathioprine	21 (6.3)	9 (6.7)	15 (8.4)	0.655
Belimumab	12 (3.5)	4 (3.0)	5 (2.8)	0.468

WBC White blood cell, *IgA* Immunoglobulin A, *IgG* Immunoglobulin G, *IgM* Immunoglobulin M, *C3* Complement 3, *C4* Complement 4, *AnuA* Anti-nucleosome antibody, *GC* Glucocorticoids (prednisolone or equivalent)

due to a lack of disease control, including upregulation in glucocorticoid (GC) dosage or the addition of new therapies, also resulted in the failure to achieve SRI-4 or maintain LLDAS.

Clinical efficacy and outcome measures

The patients were evaluated at the initial therapy (baseline) and every month thereafter. Renal involvement was defined as persistent proteinuria (>0.5 g/day or >3+if quantification not performed) or cellular casts (may be red cell, hemoglobin, granular, tubular, or mixed). The daily

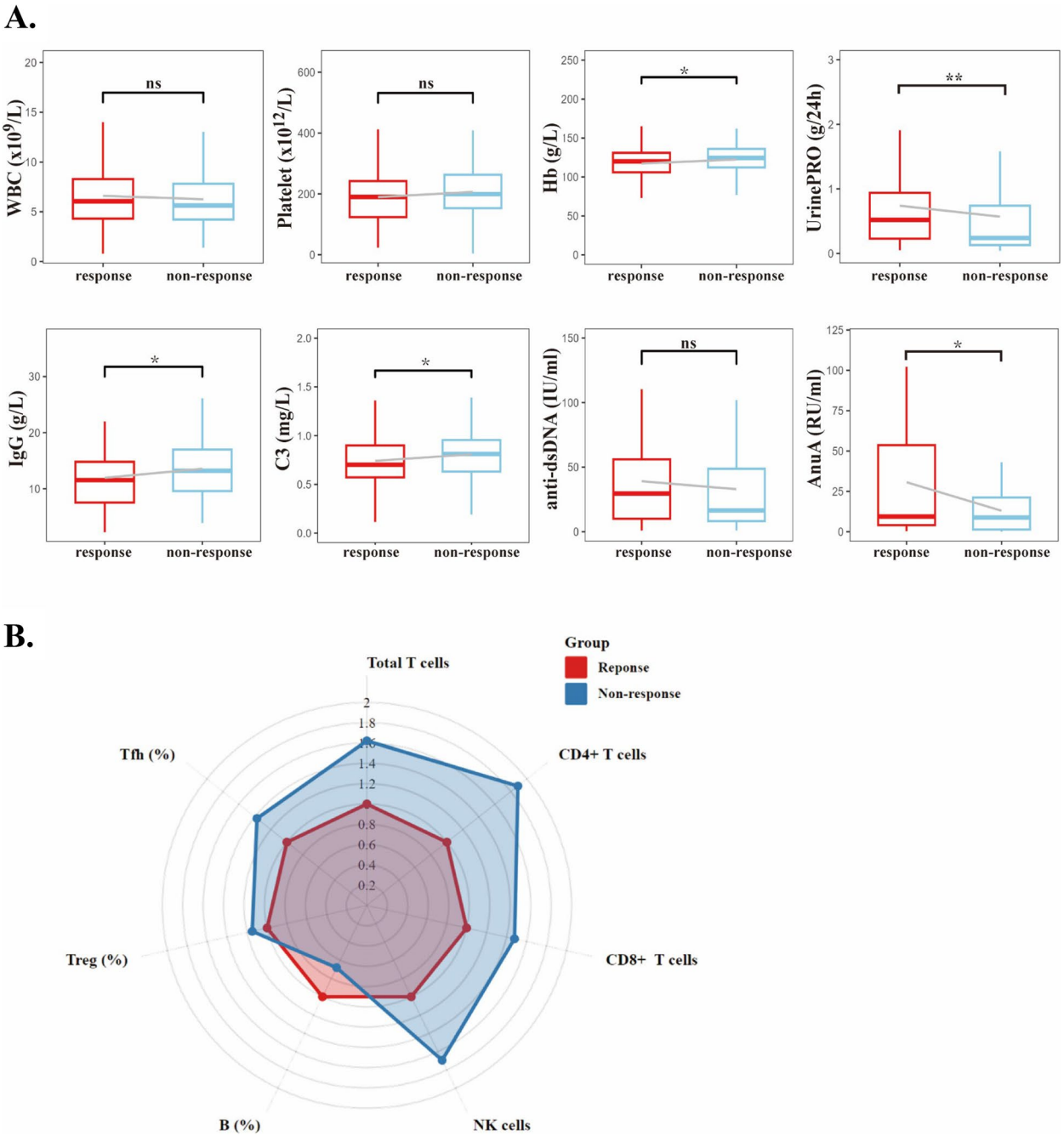

Fig. 2 Biomarkers that predict potential response to Ld-IL-2 in SLE. Patients were classified into responders (the attainment of SRI-4) and non-responders (failing to achieve SRI-4). **A** The comparison of clinical features between responders and non-responder groups presented decreased hemoglobin (Hb) and complement 3 (C3) and increased 24-h urine protein in responders. **B** Immunological parameters were analyzed as patients with lower CD4 + T cells, CD8 + T cells, and NK cells were more likely to achieve SRI-4 by Ld-IL2. *$p < 0.05$, **$p < 0.01$; WBC: White blood cell; Hb: hemoglobin; UrinePRO: Protein urine; AnuA: Anti-nucleosome antibody; Ld-IL2: low dose IL-2; SRI-4: SLE responder index-4

dose of GC was recorded at each visit. Demographic and clinical characteristics were collected including complete blood count (CBC), complete metabolic profile, urinalysis, serum immunoglobulin, complement factors C3 and C4 and anti-dsDNA antibodies were collected from medical records and hospital pharmacy prescribing database.

Flow cytometry and intracellular cytokine assays

Fresh collected whole blood was obtained and processed within 24 h. For cell staining on the surface, 100μL whole blood was incubated with the fluorophore-conjugated monoclonal antibodies (Supplementary Table 1) for 15 min in the dark, at room temperature. Then 2 ml diluted FACS Lysing Solution (BD Biosciences) was added for 10 min of erythrocyte lysis. Relative proportions of B cell, CD4 + T cell, CD8 + T cell, Treg, Tfh and NK cell subsets were acquired on a FACSAria II flow cytometer (BD Biosciences, San Jose, CA, USA) and FlowJo 10.8.1 (Becton Dickinson). Tfh cells were identified by the lineage markers $CD4^+CD45RA^-CXCR5^+PD1^+$, Tregs were defined as $CD3^+CD4^+CD25^{high}CD127^{low}$ and CLA + Tregs were defined as $CD3^+CD4^+CD25^{high}CD127^{low}$ CLA^+ (Supplementary Figure 1).

To detect the cytokine producing CD4 + T cells, peripheral blood lymphocytes were stimulated by 10 ul PMA, 10 ul ionomycin (final concentration, 750 ng/ml) and 1 ul GolgiStop in a 37℃ incubator for 5 h and then stained with human anti-CD4-APC at room temperature away from light for 30 min. Fresh fixation/permeabilization (1 ml) was used to fix and permeabilize cells. Cells were then stained intracellularly with monoclonal antibodies: BV510-IL-2, APC-TNFα, FITC-IFN-γ, PE-IL-4, and BV421-IL-17 (Supplementary Figure 2).

Statistical analysis

Descriptive variables are presented as the percentage (%), mean with standard deviation (SD), or median with interquartile range (IQR). Categorical variables were examined using the two-tailed Fisher's exact test while continuous variables were detected using the nonparametric Mann–Whitney test. Variables exhibiting a P-value < 0.10 in univariate analysis were considered for inclusion in the multivariate regression model. Factors with $P < 0.05$ in the multivariate analysis were considered significant. All statistical analyses were performed using SPSS software, version 26.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Our study enrolled 342 SLE patients treated with Ld-IL2 (mean [SD] age, 39[15] years) from Peking University People's Hospital. The baseline demographic and clinical characteristics are detailed in Table 1. Among these patients, 312 (91.2%) were female, and a median (IQR) disease duration was 8 (5–14) years. The median (IQR) SLEDAI at baseline was 8 (5–12).

Table 2 Treatment efficacy following Ld-IL2 treatment in responders and non-responders

Characteristics	Baseline	Week 12	*P* value
SELENA-SLEDAI, median (range)			
Responders	11 (8–16)	4 (2–6)	< 0.001
Non-responders	9 (6–14)	6 (4–9)	0.191
Rash, n (%)			
Responders	43 (31.6)	7 (5.1)	< 0.001
Non-responders	39 (21.9)	12 (6.7)	< 0.001
Leukopenia, n (%)			
Responders	25 (18.4)	10 (7.4)	0.034
Non-responders	33 (18.5)	18 (10.1)	0.076
Thrombocytopenia, n (%)			
Responders	31 (22.8)	10 (7.4)	0.003
Non-responders	32 (18.0)	18 (10.1)	0.100
WBC, × 109/L, median (range)			
Responders	6.13 (4.38–8.35)	6.20 (4.66–9.28)	0.285
Non-responders	5.50 (4.20–7.82)	5.92 (4.37–7.49)	0.399
Platelet, × 1012/L, median (range)			
Responders	197 (133, 251)	216 (177–286)	0.005
Non-responders	199 (153, 265)	217 (180–268)	0.069
IgA, g/L, median (range)			
Responders	2.23 (1.37, 2.87)	2.05 (1.38–2.93)	0.860
Non-responders	2.52 (1.68, 3.19)	2.40 (1.56–3.28)	0.609
IgG, g/L, median (range)			
Responders	11.60 (8.20, 15.00)	10.96 (9.13–13.04)	0.387
Non-responders	13.25 (9.60, 16.95)	11.41 (8.53–14.88)	0.045
IgM, g/L, median (range)			
Responders	0.87 (0.52, 1.33)	0.82 (0.45–1.23)	0.419
Non-responders	0.86 (0.57, 1.26)	0.83 (0.56–1.21)	0.465
C3, g/L, median (range)			
Responders	0.71 (0.57, 0.91)	0.89 (0.76–1.05)	< 0.001
Non-responders	0.81 (0.63, 0.96)	0.83 (0.70–0.98)	0.089
C4, g/L, median (range)			
Responders	0.16 (0.12, 0.21)	0.22 (0.15–0.27)	< 0.001
Non-responders	0.17 (0.12, 0.22)	0.18 (0.14–0.24)	0.106
Anti-dsDNA, IU/mL, median (range)			
Responders	28.80 (10.15, 56.00)	16.35 (9.20–35.38)	0.058
Non-responders	18.00 (8.50–56.80)	20.70 (9.35–47.25)	0.479
AnuA, IU/mL, median (range)			
Responders	16.5 (5.04, 73.66)	10.37 (3.45–26.04)	0.230
Non-responders	9.67 (1.20, 28.71)	7.33 (2.14–21.21)	0.910
UrinePRO, g/24 h, median (range)			
Responders	0.68 (0.24, 2.20)	0.38 (0.20–1.68)	0.028
Non-responders	0.26 (0.14–1.34)	0.60 (0.19–1.49)	0.194

Table 3 The changes of immunological parameters in response and non-response group

Parameters	Responders	Non-responders	*P* value
T cells (ul), median (range)	5.9 (-28.8, 138.4)	14.5 (-36.1, 47.8)	0.274
CD4+ T cells (ul), median (range)	34.1 (-11.2, 162.0)	26.9 (-24.0, 63.6)	0.037
CD8+ T cells (ul), median (range)	32.6 (-29.5, 140.6)	6.6 (-19.0, 60.8)	0.185
B cells (ul), median (range)	-17.3 (-50.6, 82.1)	24.8 (-34.8, 72.9)	0.345
NK cells (ul), median (range)	61.8 (20.8, 263.3)	59.4 (9.1, 162.2)	0.616
T cells (%), median (range)	2.8 (-5.0, 7.1)	0.3 (-4.1, 5.3)	0.716
B cells (%), median (range)	-30.9(-46.2, -12.5)	-5.1 (-30.2, 34.8)	0.008
NK cells (%), median (range)	38.4 (-13.5, 81.0)	23.0 (-8.0, 168.8)	0.961
Tfh cell (%), median (range)	-12.5 (-41.3, 56.2)	-23.4 (-37.3, 31.3)	0.485
Treg cell (%), median (range)	37.4 (-3.5, 73.0)	32.6 (10.5, 145.7)	0.470
TNF-α (%), median (range)	-2.7 (-23.8–21.9)	-26.6 (-34.5, 12.4)	0.098
IFN-γ (%), median (range)	3.7 (-30.5, 66.8)	-11.3 (-41.2, 17.0)	0.273
IL2 (%), median (range)	-0.04 (-19.4, 30.8)	-2.1 (-17.2, 34.1)	0.711
IL4 (%), median (range)	2.1 (-33.6, -54.9)	1.6 (-24.0, 70.3)	0.732
IL17 (%), median (range)	-11.1 (-36.1, 93.2)	-16.0 (-45.7, 20.1)	0.269

Comparison of clinical and immunological parameters according to efficacy response

After excluding 28 patients treated with Ld-IL2 for less than 3 months, 314 patients were included in the effective analysis and efficacy prediction. During the follow-up period, the SRI-4 response was achieved by 43.3% (136/314) after Ld-IL2 therapy for 12 weeks. To explore the potential predictors for a favorable response to Ld-IL2 treatment, 314 patients were allocated into the responder ($n=136$, mean [SD] age, 38.6 [14.7] years) or the non-responder group ($n=178$, mean [SD] age, 39.8 [14.8] years). Data showed that neutrophil ($P=0.030$), hemoglobin ($P=0.030$), IgG ($P=0.050$), C3 ($P=0.017$), and 24 h-proteinuria ($P=0.009$) reached significant differences between responder and non-responder group (Table 1 and Fig. 2A).

The improvement of clinical manifestations and laboratory parameters of patients were detailed in Table 2. Notably, a significant reduction was observed in the median (range) of SLEDAI scores from 11 (8–16) at treatment initiation to 4 (2–6) by week 12 in responders ($P<0.001$). Treatment with Ld-IL2 correlated with decreased levels of immunoglobin G (IgG) production and elevated serum C3 and C4. We also observed the significant resolution of clinical manifestations including rash ($P<0.001$), leukopenia ($P=0.034$), and thrombocytopenia ($P=0.003$) in responders group. In addition, responders also experienced a decrease in anti-double-stranded DNA (anti-dsDNA) antibody titers ($P=0.058$) and 24-h proteinuria ($P=0.028$) following Ld-IL2 treatment.

Immunological responses after Ld-IL2 treatment

A flow cytometry analysis was also conducted to investigate the potential influences of various immune cell subsets on Ld-IL2 treatment. Briefly, relatively lower CD4 + T cells, CD8 + T cells, and NK cells were associated with higher decreases in SLEDAI scores (CD4 + T cells:

Table 4 Predictors for Ld-IL2 treatment based on univariable logistic model

Variable	*P* value	OR	95%CI
Age	0.574	0.995	0.980–1.011
Disease duration	0.163	1.023	0.991–1.056
Gender	0.462	0.740	0.331–1.652
Rash	0.048	1.756	1.028–2.804
Leukopenia	0.738	1.105	0.617–1.979
Thrombocytopenia	0.186	1.458	0.834–2.551
Lymphocyte	0.072	0.980	0.958–1.002
Neutrophil	0.030	1.019	1.002–1.037
Urine protein	< 0.001		
Urine occult blood	< 0.001		
Urine cast	< 0.001	4.703	2.300–9.617
24-h proteinuria	0.127	1.166	0.958–1.419
ESR	0.056	1.020	1.006–1.034
IgG	0.038	0.956	0.908–0.998
C3	0.028	0.303	0.105–0.879
Anti-dsDNA	0.902	1.001	0.993–1.005
Anti-nucleosome antibodies	0.076	1.008	0.999–1.017
Antiphospholipid	0.925	1.002	0.963–1.042
Anti-beta-2 glycoprotein	0.681	0.998	0.991–1.006

ESR Erythrocyte sedimentation rate, *IgG* Immunoglobulin G, *C3* Complement 3

$P<0.001$, CD8 + T cells: $P=0.006$, NK cells: $P=0.002$), as was relatively higher B cells ($P=0.022$) (Fig. 2B and Supplementary Table 2). Moreover, the proportion of TNF-α, IFN-γ, IL2, IL4, and IL17 were impervious to Ld-IL2 administration (Supplementary Table 2). After adjusting for baseline values using the ratio of change to baseline, defined as [(post-treatment value minus pre-treatment value) divided by the pre-treatment value], we observed significant differences in the immune profiles of CD4 + T cells between responders and non-responders ($P=0.037$) but changes of CD8 + T cells and NK cells failed to reach significant differences (Table 3). These results suggested that patients with limited CD4 + T cells were more likely to benefit from Ld-IL2 treatment.

Predictors of Ld-IL2 treatment efficacy responses

The univariate analysis was performed to determine that neutrophil ($P=0.030$), urine protein ($P<0.001$), urine occult blood ($P<0.001$), urine cast ($P<0.001$), IgG ($P=0.038$) and C3 ($P=0.028$) as significantly associated with the failure to achieve SRI-4 (Table 4). According to multivariate analysis, rash ($P=0.026$; OR: 3.648; 95% CI: 1.170, 11.375), urine protein ($P=0.008$; OR: 2.101; 95% CI: 1.211, 3.645), urine occult blood ($P<0.001$; OR: 2.299;

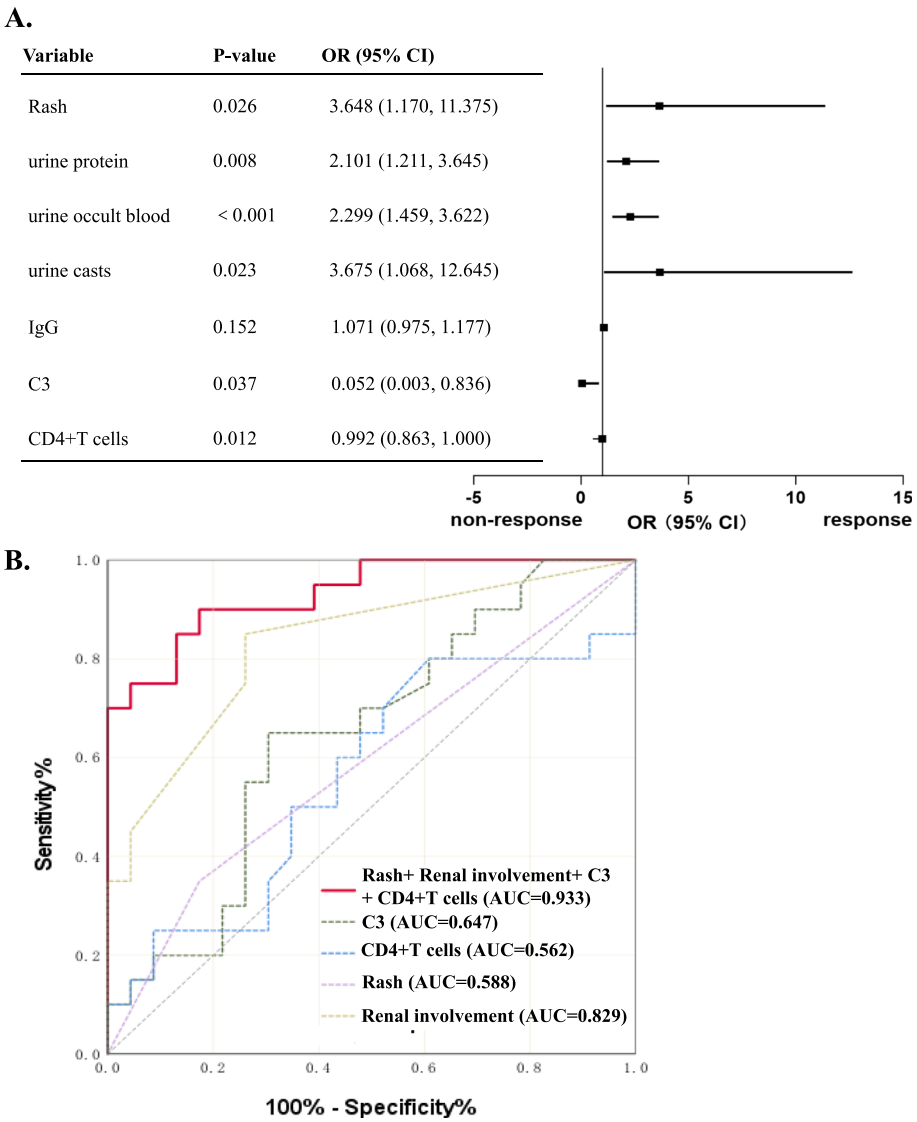

Fig. 3 Clinical indicators for the prediction of SRI-4 after Ld-IL2 treatment in SLE patients. **A** Forest plot depicting the efficacy of Ld-IL2 treatment in responder and non-responder groups. **B** Receiver operator characteristic (ROC) curve of the ability of rash, renal involvement, CD4 + T cells, and complement 3 (C3) to predict an SRI-4 response with an AUC of 0.933. Ld-IL2: low-dose IL-2; SRI-4: SLE responder index-4

95% CI: 1.459, 3.622), urine casts ($P=0.023$; OR: 3.675; 95% CI: 1.068, 12.645), C3 ($P=0.037$; OR: 0.052; 95% CI: 0.003, 0.836) and CD4 + T cells ($P=0.012$; OR: 0.992; 95% CI: 0.863, 1.000) were considered as independent and negative predictors for SRI-4 response (Fig. 3A). Notably, the ability of these clinical parameters along with immunological indicators to predict the SRI-4 response was calculated by receiver operator characteristic (ROC) curve (ROC = 0.933, Fig. 3B).

Validation of predictive markers by LLDAS

LLDAS with a GC dosage ≤ 7.5 mg/day was achieved by 35.0% (110/314) at 3 months [13]. Simultaneously, predicting the efficacy of Ld-IL2 was also validated by LLDAS. In addition to rash and lower NK cells, the analysis indicated that hematological involvements including decreased platelets ($P<0.001$; 95% CI: 2.347–17.534) and lymphocytes ($P=0.033$; 95% CI: 1.003–1.065), along with lower CLA^+ Treg ($P=0.027$; 95% CI: 1.003–1.044) emerge as potent predictors in LLDAS attainment (Table 5). In contrast, renal involvement such as urine protein and urine occult blood failed to have a significant association in achieving LLDAS (Table 5).

The concomitant effect of conventional drugs with Ld-IL2

Background therapy of SLE patients, including hydroxychloroquine (HCQ), mycophenolate mofetil (MMF), cyclosporin A (CSA), and other treatments is also generally an important determinant in achieving remission. A multivariate analysis was performed showing that MMF was more likely to reach the SRI-4 response with the combination of Ld-IL2 ($P=0.026$; 95% CI: 1.151–3.281; Fig. 4A). Additionally, based on the cut-off of GC which concurrently maximized sensitivity and specificity as 15 mg/d, Ld-IL2 contributed to a higher response rate of SRI-4 in patients with GC doses of 15 mg or more (62.90% vs 37.10%, $P=0.018$; Fig. 4B).

Persistence and safety

Among 342 patients treated with Ld-IL2 for safety analysis, sixty-nine patients discontinued after a median follow-up time of 3 (2, 4) months. Notably, the reasons behind treatment discontinuation among the 69 patients included poor tolerability for 20 (29.0%) patients, unsatisfactory recovery for 32 (46.4%) patients, failure to obtain medication due to the coronavirus disease 2019 (COVID-19) pandemic for 5 (7.2%) patients and other personal reasons for 14 (20.3%) patients. In addition, no serious adverse events were observed after Ld-IL2 treatment. The most frequent adverse effect was injection-site reactions as demonstrated by pain, redness, and swelling at injection sites in 23(6.7%) patients. Five (1.5%) patients were reported to present transient fever. There occurred 5 (1.5%) upper respiratory tract infections, 2 (0.6%) urinary tract infections, and 4 (1.2%) herpes zoster during the treatment (Supplementary Table 3).

Discussion

This study offers a comprehensive analysis of the effectiveness of Ld-IL2 in a real-world setting and first identifies predictive factors for treatment response in a large cohort of patients with SLE. These findings provide novel insights into the utilization of Ld-IL2 in SLE, emphasizing its potential role in personalized medicine.

Table 5 Validation of previous predictors by LLDAS

	The achievement of LLDAS					
	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
Rash	2.396	1.314–4.368	0.004	2.579	1.247–5.332	0.011
Platelet	4.535	2.065–9.957	< 0.001	6.414	2.347–17.534	< 0.001
WBC	0.912	0.837–0.993	0.034	0.915	0.821–1.019	0.105
Lymphocyte	1.033	1.009–1.057	0.007	1.033	1.003–1.065	0.033
Neutrophil	0.978	0.961–0.995	0.012	0.989	0.969–1.009	0.291
Hemoglobin	1.016	1.003–1.029	0.018	1.011	0.996–1.026	0.165
Platelet	1.004	1.001–1.006	0.013	1.001	0.998–1.004	0.461
Urine protein	0.696	0.522–0.927	0.013	0.887	0.618–1.272	0.514
Urine occult blood	0.548	0.352–0.853	0.008	0.660	0.385–1.130	0.130
Alanine aminotransferase	0.971	0.949–0.994	0.013	0.967	0.940–0.995	0.020
Albumin	1.078	1.034–1.123	< 0.001	1.047	1.000–1.097	0.048
NK cells (ul)	1.001	1.000–1.002	0.048	1.007	1.002–1.012	0.006
CLA^+Treg cells (%)	1.013	1.000–1.027	0.052	1.023	1.003–1.044	0.027

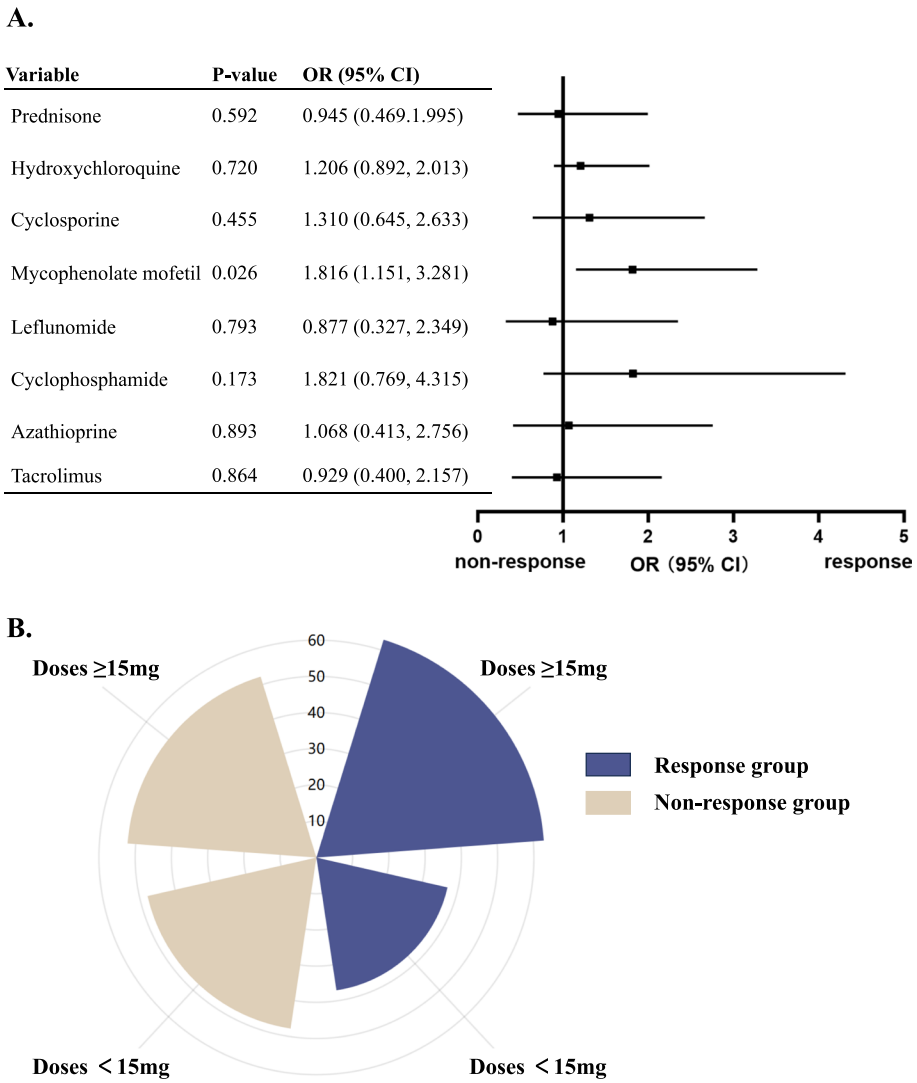

Fig. 4 The efficacy of conventional therapy synthesis with Ld-IL2 treatment. **A** Forest plot depicting the efficacy of conventional therapy with Ld-IL2 treatment. **B** The response rate in prednisone doses at least 15 mg was significantly superior to that in prednisone doses less than 15 mg. Ld-IL2: low-dose IL-2; SRI-4: SLE responder index-4

Ld-IL2 therapy has been identified to restore immune tolerance and reduce the risk of infection as previously described [2, 14]. The preferential expansion of NK cells and enhanced proliferation of CD8+ T cells, as observed in influenza-infected murine models [15, 16], aligns with our findings and underscores the therapeutic benefits of Ld-IL2 in SLE [17]. We interrogated baseline co-variates associated with disease activity and used these to predict efficacy responses for patients following Ld-IL2 treatment. Neutrophil, renal involvement, ESR, IgG, and C3 were significantly associated with the failure to achieve SRI-4. Rash, renal involvement, and C3 were considered as potent predictors for SRI-4 response consistent with previous studies [18]. The observed alterations in cell populations, particularly the reduction in CD4+T cells significantly predict clinical response to IL-2 treatment. Previous randomized controlled trials (RCTs) underscore the importance of contextualizing clinical trial results within real-world settings. Although RCTs provide high-quality evidence under controlled conditions, real-world studies are also essential for understanding Ld-IL2 performance in routine practice. The consistency of our findings with those clinical trials supports the potential of Ld-IL-2 as a viable therapeutic option for SLE, but also highlights the necessity for ongoing researches to optimize patient selection, dosing strategies, and combination therapies.

Conventional therapies for SLE usually commence with oral corticosteroids or immunomodulatory agents. However, serious adverse events and infection are common in SLE and emerge as a source of mortality, as suppression of immunity by medications obviously increases the risk of infection in SLE patients [19–21]. Our analysis of the efficacy of combining Ld-IL2 with conventional therapies reveals an intriguing aspect of SLE management. The synergistic effect observed suggests that MMF as the background therapy, together with GC doses at least 15 mg could be more effective than monotherapy. Indeed, chronic steroid therapy contributes to significant adverse effects, including increased risks of infections, osteoporosis, cardiovascular disease, and other complications. However, in our study, patients who required higher doses of GC likely had more severe or refractory disease, and the addition of Ld-IL2 significantly improves their disease activity and dosage reduction. This does not endorse chronic high-dose steroid therapy. Instead, these findings highlight the potential of Ld-IL2 to allow for better disease management, potentially offering a pathway to tapering steroids more effectively in the long term. Future research should aim to integrate Ld-IL2 to decrease corticosteroid use while maintaining remission and minimizing risks. Therefore, while chronic high-dose GC use is not desirable, Ld-IL2 may provide a beneficial adjunct to conventional therapy in patients with severe disease, with the potential to improve outcomes and ultimately reduce reliance on prolonged steroid use.

However, there still existed several limitations. First, the analysis involved multiple comparisons across various clinical and laboratory parameters, leading to a risk of type I errors, so the results especially with marginal *p*-values, should be cautiously interpreted. Additionally, statistical significance does not guarantee clinical relevance and the impact of biomarker associations on treatment efficacy must be thoroughly assessed. Furthermore, the retrospective nature of the study and the lack of a randomized control group are critical considerations. Future research should focus on prospective studies and randomized controlled trials to validate these findings. Finally, Ld-IL2 has been utilized in multiple other diseases including rheumatoid arthritis, Sjögren Syndrome, type 1 diabetes, and polymyositis or dermatomyositis [10, 11, 22, 23], and our study investigated the results from short-term follow as the median follow-up time was 3 (2, 4) months. Hence, long-term follow-up and evaluation of more diseases was required to potently validate our results.

Conclusions

Consequently, this real-world study identifies predictors such as rash, C3 and renal involvement for Ld-IL2 therapy success in SLE, underscoring the necessity of personalized treatment approaches. This finding assists physicians in making informed decisions about using Ld-IL2, thereby enhancing the management approaches for SLE.

Abbreviations

Ld-IL2	Low-dose IL-2
SLE	Systemic lupus erythematosus
SRI-4	SLE Responder Index-4
LLDAS	Lupus Low Disease Activity State
Tregs	Regulatory T cells
SD	Standard deviation
IQR	Interquartile range
WBC	White blood cell
AnuA	Anti-nucleosome antibody
ROC	Receiver operator characteristic
GC	Glucocorticoid
HCQ	Hydroxychloroquine
MMF	Mycophenolate mofetil
CSA	Cyclosporin A
COVID-19	The coronavirus disease 2019

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

J.H. conceived and designed the project. J.H., Z.L., X.Z. were in charge of participant enrollment and sample collection. R.F., X.X., B.H., K.Z., conducted data acquisition. R.F. performed data analysis. R.F. and J.H. drafted and revised the first manuscript. Y. J. provided statistical guidance and reviewed the manuscript. All the authors have revised and approved the manuscript submission.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the ethical committee of Peking University People's Hospital (approval number 2022PHB013-001) and all patients provided written consent.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

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References

1. Durcan L, O'Dwyer T, Petri M. Management strategies and future directions for systemic lupus erythematosus in adults. Lancet. 2019;393(10188):2332–43.
2. He J, Zhang R, Shao M, Zhao X, Miao M, Chen J, Liu J, Zhang X, Zhang X, Jin Y, et al. Efficacy and safety of low-dose IL-2 in the treatment of systemic lupus erythematosus: a randomised, double-blind, placebo-controlled trial. Ann Rheum Dis. 2020;79(1):141–9.
3. Rosenzwajg M, Lorenzon R, Cacoub P, Pham HP, Pitoiset F, El Soufi K, RIbet C, Bernard C, Aractingi S, Banneville B, et al. Immunological and clinical effects of low-dose interleukin-2 across 11 autoimmune diseases in a single, open clinical trial. Ann Rheum Dis. 2019;78(2):209–17.
4. Li ZG, He J, Miao M. Low-dose interleukin-2 therapy in autoimmune and inflammatory diseases: facts and hopes. Sci Bull. 2023;68(1):10–3.
5. Abbas AK, Trotta E, Simeonov DR, Marson A, Bluestone JA. Revisiting IL-2: biology and therapeutic prospects. Sci Immunol. 2018;3(25):eaat1482.
6. Guo C, Liu Q, Zong D, Zhang W, Zuo Z, Yu Q, Sha Q, Zhu L, Gao X, Fang J, et al. Single-cell transcriptome profiling and chromatin accessibility reveal an exhausted regulatory CD4+ T cell subset in systemic lupus erythematosus. Cell Rep. 2022;41(6):111606.
7. Humrich JY, Cacoub P, Rosenzwajg M, Pitoiset F, Pham HP, Guidoux J, Leroux D, Vazquez T, Riemekasten G, Smolen JS, et al. Low-dose interleukin-2 therapy in active systemic lupus erythematosus (LUPIL-2): a multicentre, double-blind, randomised and placebo-controlled phase II trial. Ann Rheum Dis. 2022;81(12):1685–94.
8. Humrich JY, Riemekasten G. Low-dose interleukin-2 therapy for the treatment of systemic lupus erythematosus. Curr Opin Rheumatol. 2019;31(2):208–12.
9. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40(9):1725.
10. Zhang X, Miao M, Zhang R, Liu X, Zhao X, Shao M, Liu T, Jin Y, Chen J, Liu H, et al. Efficacy and safety of low-dose interleukin-2 in combination with methotrexate in patients with active rheumatoid arthritis: a randomized, double-blind, placebo-controlled phase 2 trial. Signal Transduct Target Ther. 2022;7(1):67.
11. He J, Chen J, Miao M, Zhang R, Cheng G, Wang Y, Feng R, Huang B, Luan H, Jia Y, et al. Efficacy and safety of low-dose interleukin 2 for primary Sjögren syndrome: a randomized clinical trial. JAMA Netw Open. 2022;5(11):e2241451.
12. Luijten KM, Tekstra J, Bijlsma JW, Bijl M. The Systemic Lupus Erythematosus Responder Index (SRI); a new SLE disease activity assessment. Autoimmun Rev. 2012;11(5):326–9.
13. Franklyn K, Lau CS, Navarra SV, Louthrenoo W, Lateef A, Hamijoyo L, Wahono CS, Chen SL, Jin O, Morton S, et al. Definition and initial validation of a Lupus Low Disease Activity State (LLDAS). Ann Rheum Dis. 2016;75(9):1615–21.
14. He J, Zhang X, Wei Y, Sun X, Chen Y, Deng J, Jin Y, Gan Y, Hu X, Jia R, et al. Low-dose interleukin-2 treatment selectively modulates CD4(+) T cell subsets in patients with systemic lupus erythematosus. Nat Med. 2016;22(9):991–3.
15. Zhou P, Chen J, He J, Zheng T, Yunis J, Makota V, Alexandre YO, Gong F, Zhang X, Xie W, et al. Low-dose IL-2 therapy invigorates CD8+ T cells for viral control in systemic lupus erythematosus. PLoS Pathog. 2021;17(10):e1009858.
16. He J, Li Z. Dilemma of immunosuppression and infection risk in systemic lupus erythematosus. Rheumatology (Oxford). 2023;62(Suppl 1):i22–9.
17. Miao M, Xiao X, Tian J, Zhufeng Y, Feng R, Zhang R, Chen J, Zhang X, Huang B, Jin Y, et al. Therapeutic potential of targeting Tfr/Tfh cell balance by low-dose-IL-2 in active SLE: a post hoc analysis from a double-blind RCT study. Arthritis Res Ther. 2021;23(1):167.
18. Miao M, Li Y, Xu D, Zhang R, He J, Sun X, Li Z. Therapeutic responses and predictors of low-dose interleukin-2 in systemic lupus erythematosus. Clin Exp Rheumatol. 2022;40(5):867–71.
19. Hepburn AL, Davies KA. Infection and SLE. Ann Rheum Dis. 2002;61(7):668–9; author reply 669.
20. Barber MRW, Clarke AE. Systemic lupus erythematosus and risk of infection. Expert Rev Clin Immunol. 2020;16(5):527–38.
21. Kang I, Park SH. Infectious complications in SLE after immunosuppressive therapies. Curr Opin Rheumatol. 2003;15(5):528–34.
22. Rosenzwajg M, Salet R, Lorenzon R, Tchitchek N, Roux A, Bernard C, Carel JC, Storey C, Polak M, Beltrand J, et al. Low-dose IL-2 in children with recently diagnosed type 1 diabetes: a Phase I/II randomised, double-blind, placebo-controlled, dose-finding study. Diabetologia. 2020;63(9):1808–21.
23. Feng M, Guo H, Zhang C, Wang Y, Liang Z, Zhao X, Qin Y, Wu Y, Liu G, Gao C, et al. Absolute reduction of regulatory T cells and regulatory effect of short-term and low-dose IL-2 in polymyositis or dermatomyositis. Int Immunopharmacol. 2019;77:105912.

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