Therapeutic responses and predictors of low-dose interleukin-2 in systemic lupus erythematosus

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Abstract

Objective

Interleukin-2 (IL-2) is effective and well tolerated in patients with systemic lupus erythematosus (SLE). However, patient response to IL-2 therapy varies. Therefore, biomarkers are needed to efficiently identify patients who may respond well to IL-2 treatment. We investigated clinical and immunological biomarkers to predict low-dose IL-2 responses.

Methods

A pooled post-hoc analysis was performed in SLE patients who received low-dose IL-2 treatment in two clinical trials. Factors predicting responses in clinical and T-cell subset changes were evaluated by logistic regression. Good response (GR) and poor response (PR) were defined according to whether patients achieved or did not achieve an SLE Responder Index-4 (SRI-4), respectively.

Results

A good response at 68% was achieved in patients with lower Treg, compared to 0% in patients with higher Treg. In comparison to PR, GR was more strongly associated with low Treg proportions at baseline (12.85±6.07% vs. 9.43±2.82%, p<0.01). There were more patients with skin rash in the GR group than in the PR group (68.75% vs. 30.77%, p=0.042). Multivariate analysis showed that low Treg proportions and skin rash presence were both independently associated with GR to low-dose IL-2 treatment. A nomogram to identify GR probability exhibited a clear discrimination (concordance index, 0.812; 95% confidence interval, 0.64–0.97). Based on the area under the receiver operating characteristic (ROC) curve (AUC) of 0.813, the specificity of a low regulatory T cells (Tregs) proportion (≤13.35%) plus skin rash to predict GR to IL-2 therapy was 100%, with a sensitivity of 68.75%.

Conclusion

A low Treg proportion and skin rash indicate GR to low-dose IL-2 treatment in SLE patients.

Key words

systemic lupus erythematosus, low-dose interleukin-2, biomarkers

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Introduction

Systemic lupus erythematosus (SLE) is characterised by a breakdown in immune tolerance, leading to auto-reactive immune responses and consequential tissue and organ damage. Regulatory T cells (Tregs) suppress autoimmunity and regulate innate and adaptive immune responses. Treg impairment is associated with a loss of immune tolerance and autoimmunity. Low-dose interleukin-2 (IL-2) has been studied as an immunoregulatory agent that selectively promotes Treg expansion, showing promising efficacy in the treatment of SLE (1-4). However, these clinical studies showed that a substantial proportion of SLE patients had a suboptimal response to low-dose IL-2 and failed to achieve an SLE Responder Index-4 (SRI-4) at the end of their IL-2 treatment period. To date, the prediction of response to IL-2 therapy is not well studied in SLE. It was found that an increase of the CD25hi-expressing cells among $CD3^+$ $CD4^+Foxp3^+CD127lo$ Tregs might be suggestive of clinical responsiveness to low-dose IL-2 treatment (3). However, indicators evaluated after the therapy were limited in their ability to guide therapeutic decision (5). In practice, baseline biomarkers are more valuable to guide treatment choices. In this study, we analysed multiple clinical and laboratory parameters to identify baseline markers to predict response to IL-2.

Methods

Participants

The aim of this study was to analyse the therapeutic responses to low-dose IL-2 in a randomised controlled trial (RCT) (NCT02465580) of 29 SLE patients as primary cohort. Then, the prediction model was validated by using an independent cohort with 23 patients (NCT02084238). Full details of study designs and inclusion/exclusion criteria for each completed study have previously been published (1, 2). Studies were conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization Guidelines for Good Clinical Practice, and local regulations.

All the patients were treated for the first 12 weeks which included three treatment cycles with IL-2 along with standard treatment, and followed up for further 12 weeks without IL-2. Patients were evaluated at screening and every 4 weeks up to week 24. The primary efficacy endpoint was the attainment of an SRI-4 at week 12 in both trials.

The primary cohort for the development of the nomogram was the low-dose IL-2 group in trial 1, and the independent validation cohort comprised the patients with IL-2 immunological analyses in IL-2 group in trial 2. The performance of the internally validated nomogram was tested again in the validation cohort. The logistic regression formula formed in the primary cohort was applied to all patients in the validation cohort, and the total points were calculated for each patient. In this cohort, logistic regression was then performed by using the total points as a factor. Finally, the concordance index (c-index) was derived on the basis of the regression analysis.

Statistical analysis

Descriptive statistics are provided as the mean ± the standard deviation (SD), median (interquartile range (IQR) (p25 to p75)) or n (%) depending on data distribution. Differences in baseline characteristics between SRI-4 *versus* non-SRI-4 groups were compared using a Student's t-test (or, if not normally distributed, the Wilcoxon rank-sum test) and the χ^2 test for continuous and categorical data, respectively. Baseline items showing significant associations in simple (univariable analysis) models qualified for further analysis in multiple logistic regression models for the assessment of priority, independence and confounding potentiality. Difference of 24-hour urinary protein excretion (24h-UPE) during 24 weeks between low Treg group and high Treg group was compared using the pairwise comparisons with general linear model (repeated-measures ANOVA). We developed models for the prediction of SRI-4 responses using multiple imputation multivariable logistic regression. We tested the accuracy of the clinical model by comparison of the area under the receiver operator characteristic (ROC) curve (AUC) in both the primary cohort and the validation cohort. Statisti-

Table I. Univariate analysis and multivariate logistic regression analysis of the probability of an SRI-4 response in primary cohort.

Variable	SRI-4		Univariate analysis	Multivariate analysis		
	Poor responders (n=13)	Good responders (n=16)	*p*-value	RR	95% CI	*p*-value
Sex (female), n (%)	11 (84.62)	15 (93.75)	0.421	-	-	-
Age (years)	34.00 ± 12.70	30.13 ± 8.24	0.35	-	-	-
Duration (months)	20.5 (3.5, 85.5)	55 (39, 103.5)	0.13	-	-	-
SLEDAI score	11.62 ± 4.94	11.88 ± 4.03	0.88	-	-	-
Skin rash, n (%)	**5 (35.71)**	**11 (73.33)**	**0.042**	**0.08**	**0.01-0.66**	**0.02**
Routine laboratory tests						
WBC (*10^9/L)	5.43 ± 1.88	5.88 ± 3.00	0.63	-	-	-
HGB (g/L)	121.0 ± 22.10	123.0 ± 19.00	0.80	-	-	-
PLT (*10^9/L)	173.2 ± 66.07	206.7 ± 91.68	0.26	-	-	-
Lymphocytes (*10^9/L)	1.24 ± 0.57	1.35±0.80	0.69	-	-	-
24h-UPE (g/day)	0.39 (0.12, 1.8)	0.14 (0.09, 0.64)	0.20	-	-	-
Serum creatinine (μmol/L)	77.82 ± 42.96	55.70 ± 9.42	0.12	-	-	-
Uric acid (μmol/L)	347.1 ± 131.26	271.59 ± 91.84	0.11	-	-	-
GFR (ml/min)	102.47 ± 32.80	119.82 ± 12.46	0.11	-	-	-
Albumin (g/L)	38.29 ± 7.18	41.62 ± 4.08	0.19	-	-	-
ESR (mm/h)	20 (7, 44)	14 (8.5, 28)	0.20	-	-	-
CRP (mg/L)	2.38 (0.58, 8.39)	1.3 (0.53, 5.04)	0.42	-	-	-
C3 (G/L)	0.79 ± 0.32	0.83 ± 0.23	0.71	-	-	-
C4 (G/L)	0.17 ± 0.11	0.15 ± 0.04	0.53	-	-	-
Antibodies						
Anti-ANA	320 (320, 640)	320 (80, 400)	0.61	-	-	-
Anti-AnuA	43.44 (4.32, 142.8)	12.83 (0.87, 40.01)	0.16	-	-	-
Anti-dsDNA	84 (19.8, 260.4)	36.1 (18.88, 64.1)	0.13	-	-	-
Anti-Sm	1 (12.5)	3 (25)	0.33	-	-	-
Immune cells						
$CD4^+$T (% in $CD3^+$T)	45.56 ± 14.00	44.08 ± 9.62	0.75	-	-	-
$CD8^+$T (% in $CD3^+$T)	46.35 ± 15.53	48.68 ± 8.79	0.64	-	-	-
Treg (% in $CD4^+$T)	**12.85 ± 6.07**	**9.43 ± 2.82**	**0.01**	**0.70**	**0.48-1.01**	**0.055**
Teff (% in $CD4^+$T)	87.47 ± 7.19	86.93 ± 14.00	0.89	-	-	-
Th17 (% in $CD4^+$T)	15.11 ± 7.69	19.92 ± 9.13	0.14	-	-	-
Treg/Th17	**0.79 ± 0.35**	**0.51 ± 0.21**	**0.02**	-	-	-
Treg/Teff	0.15 ± 0.10	0.20 ± 0.41	0.62	-	-	-
B (% in lymphocyte)	3.71 ± 2.90	5.16 ± 5.39	0.43	-	-	-
NK (% in lymphocyte)	6.03 ± 4.96	6.39 ± 4.86	0.85	-	-	-
Treg (cells/μl)	13.96 ± 7.9	12.81 ± 7.11	0.69	-	-	-
Cytokines						
IFN-α (pg/ml)	5.28 (2.92, 10.49)	4.74 (2.67, 9.35)	0.30	-	-	-
IL-2 (pg/ml)	1.85 (1.32, 2.95)	2.07 (1.64, 3.24)	0.27	-	-	-
IL-21 (pg/ml)	1.68 (0.96, 4.85)	1.55 (0.27, 3.29)	0.43	-	-	-
IFN-γ (pg/ml)	1.06 (0.78, 1.31)	1.01 (0.72, 1.23)	0.49	-	-	-
IL-17 (pg/ml)	0 (0, 0.61)	0 (0, 0.03)	0.19	-	-	-
IL-10 (pg/ml)	2.75 (2.07, 3.94)	2.85 (2.13, 3.85)	0.89	-	-	-
TGF-β (pg/ml)	50870 (26736, 75191)	60131 (26848, 92819)	0.51	-	-	-

Data are presented as the median (IQR) or the mean ± standard deviation (SD) for continuous variables and as the count (percentage) for categorical variables. Good responders were defined as patients who achieved a Systemic Lupus Erythematosus (SLE) Responder Index-4 (SRI-4), while poor responders were defined as patients who did not achieve an SRI-4.

CI: confidence interval; SLEDAI: SLE Disease Activity Index; WBC: white blood cell; HGB: haemoglobin; PLT: platelet; 24h-UPE: urine protein excretion per 24 hours; GFR: glomerular filtration rate; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; C3: complement 3; C4: complement 4; Anti-AnuA: anti-nucleosome antibody; Anti-dsDNA: anti-double-stranded DNA antibody; Treg: regulatory T cell; Teff: effector T cell; Treg#: absolute number of Tregs; IFN-α: interferon-α; IL-2: interleukin-2; IL-21: interleukin-21; IFN-γ: interferon-γ; IL-10: interleukin-10; TGF-β: transforming growth factor-β; ANA: Anti-nuclear antibody; Sm: Anti-Sm antibody.

cal analyses were performed using SPSS v. 22.0 or R v. 3.6.3 software. Two-sided *p*-values <0.05 were considered statistically significant.

Results

Characteristics of patients and comparisons of responses to IL-2 therapy

Characteristics of the patients in the primary and validation cohorts are shown in Supplementary Table S1. Patients were divided into those with good response (GR) and those with poor response (PR) according to whether they achieved or did not achieve an SRI-4, respectively, at week 12. Sixteen patients (55.17%) had GR to low-dose IL-2 treatment, whereas 13 patients had PR.

In comparison to PR, GR was more strongly associated with low Treg proportions (12.85±6.07% *vs*. 9.43±2.82%, *p*=0.01) and low Treg/Th17 ratios at baseline (0.79±0.35% *vs*. 0.51±0.21%, *p*=0.02) (Table I). There were more patients with skin rash in the GR group (68.75%, 11/16) than in the PR group (30.77%, 4/13) (*p*=0.042) (Table I).

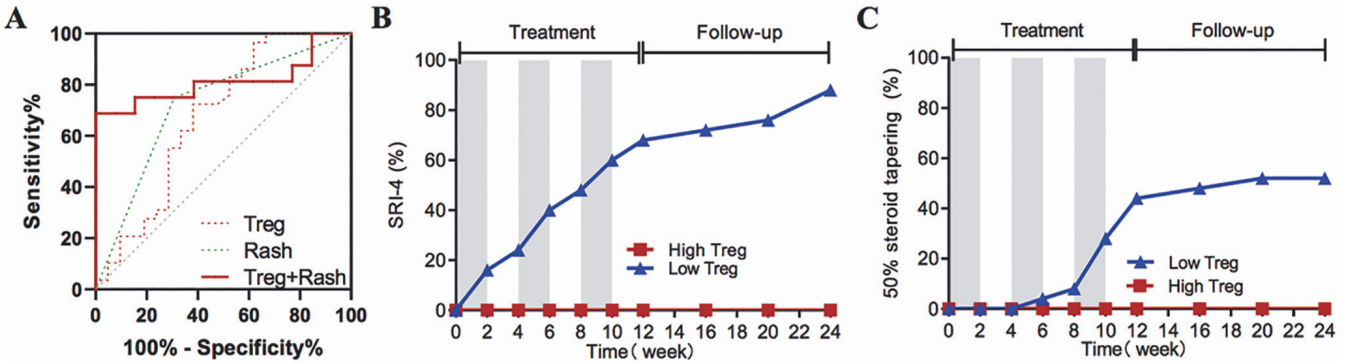

Fig. 1. Treg proportion and rash for the prediction of week-24 SRI-4 in primary cohort. (**A**) Receiver operator characteristic (ROC) curve of the ability of the proportion of Tregs to predict an SRI-4 response. Time until achieving an SRI-4 (**B**) or 50% steroid tapering (**C**) by initial disease presentation.

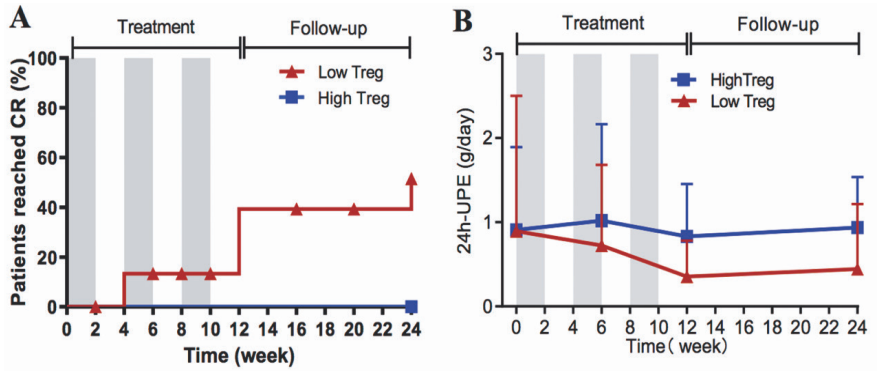

Fig. 2. Indication of clinical characters by Treg in primary cohort. Change of patients achieved CR (**A**) and 24h-UPE (**B**) during 24 weeks by initial disease presentation. CR, complete renal remission. 24h-UPE, urine protein excretion per 24 hours.

A multivariate logistic regression analysis was performed using all factors associated with GR in the univariate analysis. A lower Treg proportion and skin rash were independently associated with GR to low-dose IL-2 treatment with a marginally significant *p*-value (p=0.055 and p=0.02, respectively (Table I).

Low Treg proportion predicts clinical response to IL-2 treatment

We divided the SLE patients who received IL-2 treatment into two groups, *i.e.* those with a low or high proportion of Tregs, according to a cut-off value of 13.35%, calculated by ROC with an AUC of 0.73 (Fig. 1A). The subsequent analysis suggested that patients with a low proportion of Tregs had a three times higher probability of achieving GR to IL-2 treatment than patients with a high proportion of Tregs (p=0.011) (Table II).

A good response at 68% of patients was achieved in the low Treg group at week 12, compared to 0% in the high Treg group. In the follow-up period, 88% of patients in the low Treg group achieved GR at week 24 at end of the follow-up, compared to 0% in the high Treg group (Fig. 1B). The proportion of patients who achieved complete renal remission (CR) was significantly higher in the low Treg group than in the high Treg group at week 12 (33.33% *vs.* 0%) and week 24 (40% *vs.* 0%) (Fig. 2A). The low Treg group had a lower 24h urine protein excretion (24h-UPE) at week 12 than the high Treg group (0.35±0.43 g/day *vs.* 0.83±0.62 g/day, p=0.052) (Fig. 2B). The proportion of patients achieved 50% steroid tapering was also significantly higher in the low Treg group than that in the high Treg group at week 12 (44% *vs.* 0%) and week 24 (52% *vs.* 0%) (Fig. 1C).

Modelling and validation

To further clarify the application of these predictive markers, a nomogram was developed to estimate the likelihood of GR to low-dose IL-2 treatment in each individual patient (Fig. 3). The adjusted c-index for predicting GR in the prima-

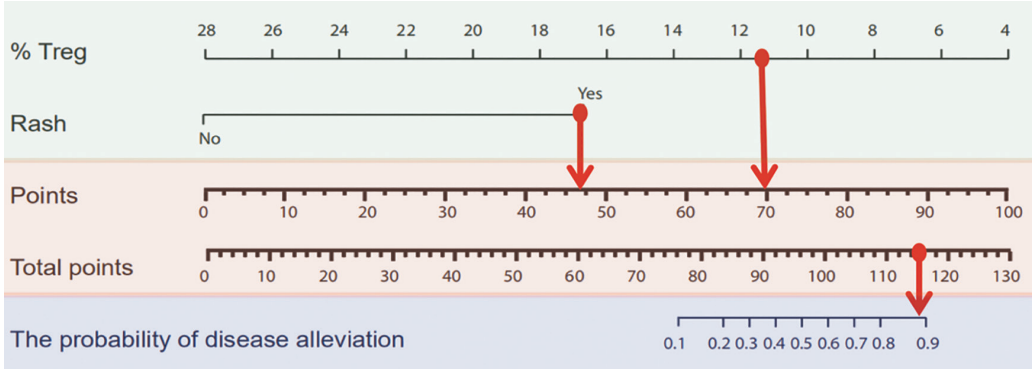

Fig. 3. Nomogram for the prediction of clinical remission following low-dose IL-2 therapy in primary cohort. To use the nomogram, an individual patient's value is located on each variable axis, and a line is drawn upward to determine the number of points received for each variable's value. The sum of these numbers is located on the total points axis, and a line is drawn downward to the axis for the probability of disease alleviation to determine the likelihood of an SRI-4.

Table II. The indication of Treg on SRI-4 in primary cohort.

		Poor responders SRI-4(-)	Good responders SRI-4(+)	*RR*	*p*-value
Treg	≤13.35	8	16	3	0.011
	>13.35	5	0		

SRI-4: Systemic Lupus Erythematosus Responder Index-4; RR*:* relative risk.

ry cohort was 0.812, ranging from 0.64 to 0.97. In the independent validation cohort, the c-index was 0.776, ranging from 0.49 to 1.06 and thus showing good discrimination ability across all patients. The calibration curves of the nomogram for the probability of SRI-4 demonstrated good agreement between prediction and observation in the primary cohort and the validation cohort (Suppl. Fig. S1). Besides, the AUC for the combined model (*i.e.* the proportion of Tregs and the presence of skin rash) was 0.813, with the sensitivity of 68.75% and 100% specificity (Fig. 1A), which was also validated in the validation cohort (Suppl. Fig. S2).

Discussion

$CD4^+$ Tregs play a central role in immune tolerance, and Treg dysfunction is well described in autoimmune disorders. *In vivo,* Treg expansion through low-dose IL-2 treatment has been reported in a number of diseases, including chronic graft-*versus*-host disease (cGVHD), type 1 diabetes, SLE and several other autoimmune diseases (AIDs) (1-4, 6-8). These studies indicated that this treatment is well tolerated and effective, but prediction of patients who may have a better response to low-dose IL-2 is still a challenge in clinical practice.

Previous studies showed low Tregs and IL-2 in the circulation of SLE patients and the efficacy of low-dose IL-2 treatment. In this study, we proved that a low proportion of Tregs and the presence of skin rash could be predictors of responses to low-dose IL-2 therapy in SLE patients.

An increase of CD25hi-expressing Tregs was associated with clinical responsiveness to low-dose IL-2 (3). However, post-treatment indicator would not help decision making by clinicians. Our findings in this study support the utility of the baseline Treg and skin rash before IL-2 administration to predict SRI-4 response. We also built a predictive model, which may help clinicians in decision making. The model indicated that Treg ≤13.35% plus skin rash presence predicted GR to IL-2 therapy with 100% specificity and 68.75% sensitivity. In addition, there was a trend that serum IL-2 and IL-10 levels were decreased in the SRI-4 responder group, although not statistically significant. This difference may become statistically significant with a larger sample size.

It remains unclear why there is a subgroup appearing to benefit more from low-dose IL-2. Low number of Treg cells was found in skin lesion of patients with cutaneous erythematosus (9). Low-dose interleukin-2 specifically expands Treg cells to confer immunosuppressive capacity to autoimmune response and inflammation. Treg deficiency can cause autoimmune skin diseases including vitiligo, alopecia areata, psoriasis, systemic sclerosis and SLE (10). Therapies to restore the Treg cells can provide local immune tolerance for autoimmune skin disorders (10). Treg cells reduce inflammation severity by restraining type I interferons (IFN-I) in a mouse model. Depletion of Treg cells induces IFN-I and IFN-stimulated gene expression, and leads to accumulation of $CD8^+$ T cells in lesional skin. Mononuclear phagocytes were the source of IFN-I, and their depletion reversed the effect of Treg cell depletion (11).

The limitations of this study included the small sample size and unequal allocation ratio. The cut-off of Treg >13.35% leaves only 5/29 in the high Treg subset. Compare with healthy subjects, Treg cells in SLE are significantly lower, leading to a low proportion of high Treg group in SLE (12). Therefore, a large sample size would be required to confirm the results in this study.

Conclusion

In summary, we found in a pooled *post-hoc* analysis that a low Treg proportion and skin rash indicated good response to low-dose IL-2 treatment in SLE patients.

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