SUPPLEMENTARY METHODS FOR

Leflunomide Versus Azathioprine for Maintenance Therapy of Lupus Nephritis: A Prospective, Multicenter, Randomized Trial and Long-term Follow-up

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1. Primary Objectives

To compare the efficacy and safety of leflunomide (LEF) and azathioprine (AZA) in long-term maintenance therapy for subjects with lupus nephritis (LN).

2. Study Design

This is a randomized, open, parallel-controlled, multicenter clinical study.

a. Induction therapy period: Subjects with active LN will be treated with the NIH regimen (cyclophosphamide (CYC) + Pred) for 6–9 months.

CYC: Intravenous infusion, 0.5–1 g/m^2 body surface area, once a month, a total of seven times; Prednisone: Oral. During the 1st month, 1 mg/kg/d; starting at the 2nd month, reduce by 5 mg every 2 weeks; after reducing to 30 mg/d, lower the amount of reduction to 2.5 mg every 2 weeks; at the end of 6 months, the prednisone dose should not exceed 10 mg/d (regarding the specific method of hormone reduction during the induction period, the attending physician can adjust the dose according to the subject's specific urine protein and kidney function level). If necessary, induction therapy would be extended to 9 months for those who showed inadequate clinical response after 6 months of treatment.

b. Maintenance therapy period: After remission induction therapy, subjects who achieved partial response (PR) or complete response (CR) will be randomized to one of two treatment arms in a 1:1 ratio with different maintenance of remission treatment regimens (AZA + Pred referred to as the AZA group or LEF + Pred referred to as the LEF group) by the central random principle (network random system program). After 6 months of remission induction therapy, if subjects do not achieve CR or PR, they can continue the original treatment regimen for an additional 3 months. If CR or PR is achieved after the additional 3 months, subjects will be randomized to either of the two groups (1:1, AZA group and LEF group). If remission is still not achieved after a total of 9 months of induction therapy, the patients will not be enrolled in this study. The maintenance of remission period is 36 months.

c. Treatment regimens:

1) AZA group: azathioprine, oral, 1.5-2 mg/kg/d (maximum dose is 100 mg/d), initial dose is 50 mg/d (if no abnormality is detected by weekly blood tests, then increase to 100 mg/d at the 2nd month and maintain the dose until the end of the study if no adverse events occur. If any adverse event occurs, the dose will be reduced as appropriate until the end of the study).

2) LEF group: leflunomide, oral, 20 mg/d.

During the maintenance period, immunosuppressants can be combined with glucocorticoids in both groups, but the prednisone dose should not exceed 10 mg/d. During the 9th–12th month of the maintenance period, the glucocorticoid will be gradually reduced to (equivalent to prednisone) 7.5 mg/d, and during the 12th–15th month of the maintenance period, the dosage equivalent to prednisone will be 5–7.5 mg/d until the end of the experiment.

During the follow-up period, if severe extrarenal symptoms occur, the glucocorticoid dose can be increased (equivalent to prednisone, 1 mg/kg/d) for no more than 2 weeks and gradually reduced thereafter.

3. Sample Size

This study was designed as a non-inferiority trial. The non-inferiority margin was set at 12% for the primary outcome (flare at 36 months), meaning that the lower bound of the two-sided 95% confidence interval for the difference in flare rates between the LEF and AZA groups (as reference) should exceed −12%. A previous study in patients with SLE reported flare rates of 15% in the LEF arm and 20% in the AZA arm. Assuming that the flare rates in LEF and AZA groups at 36 months will differ by 5%, a sample size of 158 patients was needed to yield a power of 80% and establish the non-inferiority of LEF to AZA, with a one-sided α level of 0.025. The sample size calculation made the conservative assumption that the dropout rate would be as high as 20%. Therefore, the required sample size is 200.

Non-Inferiority Tests for the Difference Between Two Proportions

Numeric Results for Non-Inferiority Tests for the Difference Between Two Proportions

Test Statistic: Z-Test with Unpooled Variance

H0: P1 - P2 ≥ D0 vs. H1: P1 - P2 = D1 < D0.

Target Power	Actual Power*	N1	N2	N	Ref. P2	P1|H0 P1.0	P1|H1 P1.1	NI Diff D0	Diff D1	Alpha
0.80	0.80276	101	101	202	0.2000	0.3000	0.1500	0.1000	-0.0500	0.025
0.80	0.80457	79	79	158	0.2000	0.3200	0.1500	0.1200	-0.0500	0.025
0.80	0.80404	57	57	114	0.2000	0.3500	0.1500	0.1500	-0.0500	0.025

* Power was computed using the normal approximation method.

4. Study Period and Follow-up Timing

The study period is 42–45 months.

Follow-up time:

a. Induction period: Follow-up will be performed at the screening day, enrollment day, 2nd week after enrollment, and 1st, 2nd, 3rd, and 6th month after enrollment (after 6 months of remission induction therapy, if subjects do not achieve CR or PR, they will continue the original regimen, and follow-up will be performed at the 9th month). After the start of treatment, periodic disease assessment and curative effect evaluation will be carried out every 3 months.

b. Maintenance period: During the 1st month of the maintenance period, follow-up will be performed every 2 weeks. From the 2nd to 4th month of the maintenance period, the follow-up frequency will be reduced to once a month and then continuously reduced to every 2 months. During the first 12 months of treatment, the periodic condition assessment and curative effect evaluation will be carried out every 2 months, followed by every 4 months until the end of the study.

5. Medication and Usage

Test drug:

a. Leflunomide Tablets (10 mg/tablet, Suzhou Changzheng-Cinkate Pharmaceutical Co., Ltd.)

b. Cyclophosphamide Powder for the Injection Solution (0.2 g/vial, no restrictions on manufacturers)

c. Azathioprine Tablets (50 mg/d, Sine Pharmaceutical General Factory, which belongs to Shanghai Pharmaceutical (Group) Co., Ltd.)

d. Prednisone Tablets (5 mg/tablet, Sine Pharmaceutical General Factory, which belongs to Shanghai Pharmaceutical (Group) Co., Ltd.)

6. Concomitant Medication

Antihypertensive drugs, such as β receptor blockers and calcium channel blockers, should be used appropriately (the target systolic blood pressure is below 140 mmHg). Angiotensin-converting-enzyme inhibitors and angiotensin II receptor blockers can be added as second-line antihypertensive drugs for patients who newly developed hypertension during the follow-up. If they have been used by subjects before enrollment, the original regimen can be sustained in principle.

The use of drugs to treat other diseases is permitted and must be recorded.

Both groups can use hydroxychloroquine (maximum dose ≤400 mg/d).

Immunosuppressants other than CYC, leflunomide, and AZA are not allowed in either group.

7. Inclusion/Exclusion Criteria; Discontinuation and Withdrawal of the Study

a. Inclusion criteria:

1) Aged 18–65 years;

2) A clinical diagnosis of systemic lupus erythematosus (SLE) according to the 1982 SLE diagnostic criteria of the American College of Rheumatology;

3) Systemic lupus erythematosus disease activity index (SLEDAI) score ≥8;

4) Within 90 days of baseline (Day 0), have a biopsy-proven diagnosis of active LN, with a pathological classification of class III or IV active or active/chronic LN (concomitant class V is permitted) and class V LN (International Society of Nephrology/Renal Pathology Society 2003);

5) Continuous proteinuria (≥1 g/24 h) with or without microscopic hematuria;

6) Signed the informed consent forms.

b. Exclusion criteria:

1) Known to be allergic to LEF, CYC, and AZA;

2) Subjects who have used cytotoxic drugs, such as CYC, within 90 days of baseline (Day 0) or received more than 200 mg methylprednisolone pulse therapy within 6 weeks of baseline (Day 0);

3) Weight <45 kg;

4) Serious infection and other fatal complications;

5) Severe lupus activity, such as neuropsychiatric systemic lupus erythematosus;

6) Extensive crescentic nephritis (>50%) with significantly abnormal kidney function;

7) A history of active gastric ulcer or active inflammatory gastrointestinal disease within 6 months of baseline (Day 0);

8) Subjects with obvious blood system diseases and abnormal laboratory examination (white blood cell (WBC) count $<3 \times 10^9$/L or platelet (PLT) count $<50 \times 10^9$/L, except that caused by SLE);

9) Moderate to severe anemia;

10) A history of chronic hepatitis;

11) Active tuberculosis;

12) Abnormal liver function (alanine transaminase (ALT) or aspartate aminotransferase (AST) >2 times higher than the upper limit of normal, except that caused by SLE);

13) Abnormal kidney function with estimated glomerular filtration rate <30 mL/min/1.73 m^2;

14) A history of alcoholism within 2 years;

15) A history of a malignant tumor, except skin and cervical intraepithelial neoplasia;

16) Decompensated cardiac insufficiency or severe hypertension;

17) Psychiatric subjects;

18) Epilepsy and other disorders of the nervous system;

19) Pregnant women, lactating women, or subjects who are unwilling to take effective contraception measures;

20) Other connective tissue diseases;

21) Subjects who need >1 mg/kg/d prednisone to control extrarenal lesions;

22) Patients with poor drug compliance.

c. Discontinuation of the study:

1) Occurrence of serious adverse events (WBC count <2000/mm^3 or PLT count <50000/mm^3);

2) Occurrence of severe gastrointestinal adverse events, subjects who cannot tolerate the agent despite adjusting the treatment (such as reducing the drug dose);

3) Progressive decline in kidney function: SCr doubling or progressing to kidney failure;

4) Occurrence of fatal complications, such as lupus encephalopathy or severe infection;

5) Pregnancy;

6) Unwilling to continue treatment or poor drug compliance;

7) During the induction period, the subjects' disease progresses, requiring high-dose glucocorticoid treatment (equivalent to a prednisone dose >1 mg/kg/d for more than 2 weeks) or other immunosuppressants to control the disease or remains no response after 9 months of treatment;

8) During the maintenance of remission period, one of the following occur:

i. Recurrent LN requiring high-dose glucocorticoid therapy (>30 mg/d);

ii. Recurrence of extrarenal symptoms requiring the use of high-dose glucocorticoid (equivalent to a prednisone dose >1 mg/kg/d for more than 2 weeks) or other immunosuppressants to control the disease;

iii. Subjects with LN who experience proteinuria flare and/or moderate to severe kidney flare.

d. Withdrawal:

Subjects can withdraw from the trial at any time. The investigator may also discontinue the treatment of subjects for a variety of reasons (see trial discontinuation criteria), including adverse events, safety considerations, poor or no efficacy, or the subjects' inability to comply with the protocol.

8. Observation Items

a. Clinical indicators: General condition (such as weight, blood pressure, heart rate, and pulse) and disease-related characteristic clinical manifestations and signs. For women, menstruation will also be observed. For each follow-up, the above data will be recorded.

b. Lab test:

1) Routine blood tests, WBC, hemoglobin, and PLT examined at each follow-up;

2) Routine urine + urinary sediment microscopy examined at each follow-up;

3) 24-h urine protein quantity examined during the induction period (once at baseline and once a month within the first 3 months, followed by once every 3 months) and the maintenance period (once a month during the first four months, followed by once every 2 months);

4) Liver function: At least including ALT, AST, albumin, and total bilirubin examined at each follow-up;

5) Kidney function: At least including SCr, blood urea nitrogen, and eGFR examined during the induction period (once at baseline and once a month during the first 3 months, followed by every 3 months) and the maintenance period (once a month during the first 4 months, followed by every 2–4 months);

6) Immunological examination: At least including antinuclear antibodies (ANA) and anti-ds-DNA examined during the induction period (once at baseline and once at the 3rd, 6th, and 9th month) and the maintenance period (once every 2 months during the first 12 months and every 4 months thereafter);

7) Complement: At least including C3 and C4 examined during the induction period (once at baseline and one time at the 3rd, 6th, and 9th month) and the first 12 months of the maintenance period (examined once every 2 months and every 4 months thereafter);

8) Erythrocyte sedimentation rate examined during the induction period (once at baseline, once a month during the first 3 months, and once every 3 months thereafter) and the maintenance period (once a month during the first 6 months, followed by once every 2 months);

9) Electrocardiogram: once before treatment.

9. Efficacy Evaluation Criteria

a. Induction therapy:

1) Complete response (CR, all the following conditions should be met simultaneously):

i. 24-h urine protein quantity <0.5 g;

ii. Inactive urinary sediment (RBC <5/high-power field (HPF), WBC <5/HPF);

iii. Serum albumin ≥35 g/L;

iv. Improved or stabilized kidney function (SCr change is within ±25% of baseline value).

2) Partial response (PR, all the following conditions should be met simultaneously):

i. Significant improvement in 24-h urine protein (at least a 50% decrease in the 24-h urine protein to <3 g/24 h if the baseline urine protein is >3.5 g/24 h, or to ≤1 g/24 h if the baseline urine protein does not reach the level of nephrotic syndrome);

ii. Serum albumin ≥30 g/L;

iii. Stable or improved kidney function (SCr change within ±25% of baseline value).

3) No response (subjects are eligible if they meet any one of the following criteria):

i. Continuous urine protein, 24-h urine protein quantity ≥3 g or decreased by <50% compared with baseline;

ii. Progressive impairment of kidney function (compared with baseline, SCr increased >50 μmol/L or a decrease in the creatinine clearance rate by >15%);

iii. Early discontinuation or withdrawal from the trial due to adverse drug events.

b. Maintenance of remission in subjects who achieved CR or PR

1) Kidney flares:

(i) the recurrence or development of nephrotic syndrome (24-h proteinuria ≥3.5 g and serum albumin <30 g/L), (ii) abnormal kidney function (>30% increase in SCr within a 1-month period directly attributed to lupus and confirmed 2 weeks later, or (iii) 2-fold increase in proteinuria (24-h proteinuria >1 g in patients with proteinuria <0.5 g/24 h (CR) at the end of induction or doubling of the proteinuria in patients with PR at the end of induction). A kidney flare may occur with or without new or increased hematuria (≥5 RBC/HPF) or the appearance of cellular casts.

2) Extrarenal flares

Disease activity of extrarenal organs or systematic disease activity occurs with a SLEDAI score ≥10.

Note: Flares must be re-checked 2 weeks after the initial examination to validate the diagnosis.

10. Randomization and masking

Patients fulfilling the inclusion/exclusion criteria were allocated to LEF or AZA group by randomization. Randomization was performed using a computerized, interactive voice-response system with stratification according to center, age, gender, and kidney biopsy classification. This is an open label study without masking.

11. Endpoints and Statistical Indicators

a. Endpoints:

1) Primary efficacy endpoint: time to kidney flare.

2) Secondary efficacy endpoint: the number of patients achieving complete kidney response (proteinuria <500 mg per 24 h, absence of hematuria and cellular casts, and improved or stable SCr within ±25% of baseline); kidney -associated variables, including 24-h proteinuria, SCr, and serum albumin over time; frequency of extrarenal flares; immunologic variables (C3, C4, and anti-double-stranded DNA antibodies); and safety profile.

b. Statistical indicators:

1) Main indicators: kidney flare time and kidney flare-up rate.

2) Other indicators:

i. Extrarenal flare rate and extrarenal flare-up time

ii. Incidence rate of SCr doubling

iii. Incidence rate and timing of the composite endpoint (kidney failure or death)

iv. Incidence rate of adverse events and serious adverse events

v. Rate of withdrawal in maintenance phase

vi. SCr

vii. 24-h urine protein

viii. Serum albumin

ix. ANA positive rate or titer

x. Positive rate or titer of anti-dsDNA

xi. Complement C3, C4

xii. SLEDAI

12. Adverse Events

For all observed or spontaneously reported adverse events, adverse event reports should be filled in and submitted, and the correlation between the adverse event and the drug should also be determined.

a. Definition of adverse events: Adverse medical events in subjects during clinical trials that are not necessarily causally related to drug use or treatment.

b. Adverse events include, but are not limited to:

1) Abnormal laboratory findings;

2) Symptoms and signs with clinical significance;

3) Overdose;

4) Drug withdrawal;

5) Drug abuse;

6) Drug misuse;

7) Drug dependence;

8) Pregnancy events.

c. A serious adverse event refers to any adverse medical events occurring at any dose that includes any of the following conditions:

1) Resulting in death;

2) Life-threatening;

3) Require hospitalization or the original length of hospitalization extended;

4) Cause permanent or severe disability/incapacity to work;

5) Resulting in congenital deformity/birth defects.

d. Evaluation of adverse events:

The correlation between adverse events and medication will be judged according to the following 5 grades: positive, probable, probable, unknown, and irrelevant. The first three grades are classified into adverse reactions.

The degree of adverse reaction is divided into three grades:

Grade 1(+): Mild: subjects recover within a short time without treatment, and subjects take medication as usual;

Grade 2(++): Moderate: Symptoms are more obvious, and subjects can continue the drug after temporary drug withdrawal or treatment;

Grade 3(+++): Reaction is severe, and subjects must discontinue the drug.

e. Principles for the management of major adverse events:

1) Abnormal hemogram and liver function:

	The original plan continuation	Dosage reduction	Drug withdrawal
Hemogram	WBC (white blood cell) count≥3×10^9/L	WBC count <3×10^9/L	WBC count <2×10^9/L
Liver function	Transaminase increased <1.5 times	1.5 times ≤ transaminase elevation <3 times	Elevated transaminase ≥ 3 times

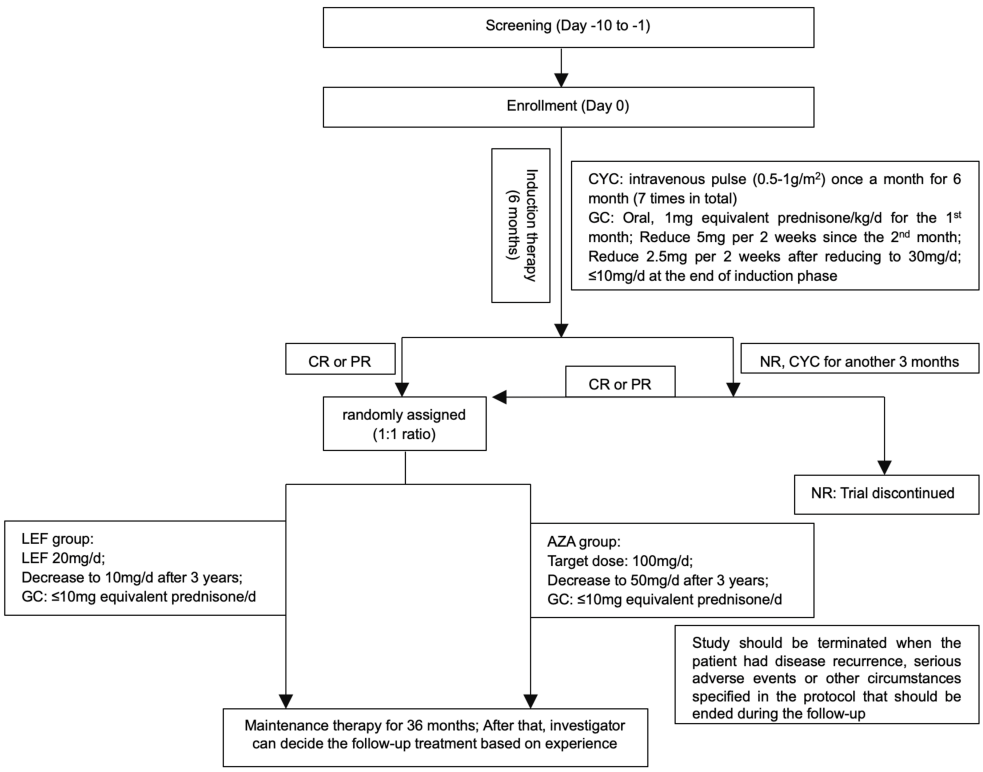
In the above cases, hepatoprotective drugs and WBC-elevating drugs can be added as appropriate. The investigator will adjust the regimen according to the correlation between the adverse events and the drug. If reexamination shows that liver function is normal, the original treatment dose can be restored. The above treatment can be repeated three times. If the liver function is abnormal for the 4^{th} time after dosage reduction/drug withdrawal, the dosage should not be increased/restored.

2) Drug allergy: withdraw from the study and be treated based on clinical experience.

3) Other adverse events: be treated according to clinical diagnosis and treatment routine. For subjects with serious adverse events, they may withdraw from the study at the discretion of attending physicians.

13. Protocol Flow Chart

Protocol flow chart (without considering the adjusted contents of the Protocol):



14. Data Collection and Management

The researcher is responsible for maintaining accurate, complete, and up-to-date records for each subject. The researcher is also responsible for maintaining any source files related to the research, including any photos, movies, tracings, computer CDs, or tapes.

Documents that identify subjects beyond the subject number will not be submitted to the sponsor (for example, signed informed consent documents or initials of the subject's name) and must be kept strictly confidential by the investigator (unless it is necessary to allow regulatory agencies to conduct audit scopes), research supervisor, or sponsor representative. On-site personnel will use the electronic data collection (EDC) system provided and approved by the sponsor to record all data of each research subject through the electronic case report form (eCRF). The research center must complete the eCRF in time, and the researcher must check the completed eCRF in time after each visit for each subject.

The EDC system automatically generates queries through computer checks embedded in the system to ensure the accuracy, quality, consistency, and completeness of the database. Manual queries generated by the review by monitors, medical coders, and other data management personnel are also generated and tracked within the EDC system. The site will resolve the query and correct the entered data when

necessary. Every change to the data is captured in the EDC system audit trail. After the research is completed, or after reaching the pre-designated point in the research, the data management will lock the database and generate the dataset required for data analysis and reporting.

15. Data and Analysis

Descriptive analysis is performed for the general characteristics of patients. Continuous variables with a normal distribution and non-normal distribution are expressed as the mean ± standard deviation (mean ± SD) and median and interquartile range (median, IQR), respectively. The grade data are compared by Ridit analysis, and the adverse event (AE) rate and count data are compared by the chi-square test or Fisher's exact test. The measurement data are compared by Student's t-test. Time to flare, the remission time, kidney survival time, and survival time of subjects between the two groups are analyzed by survival analysis. The survival curve is analyzed by the Kaplan–Meier method.

16. Study approval

This study was approved by Shanghai Renji Hospital Ethics Committee (No. 2010-8) and all participants provided written informed consent. The study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

17. Patient and public involvement

At what stage in the research process were patients/the public first involved in the research and how?

A: Lupus nephritis patients were involved in this research from the beginning of the study. After 6–9 months of the intravenous cyclophosphamide regimen combined with glucocorticoids, patients achieved complete or partial response (CR or PR) were randomly assigned to the leflunomide group or azathioprine group for a 36-month maintenance therapy.

How were the research question(s) and outcome measures developed and informed by their priorities, experience, and preferences?

A: Patients were involved in the original research and actively contributed to identifying the issue of inconsistent reporting, the need for guidance, and the research question.

How were patients/the public involved in the design of this study?

A: Patients/the public were not involved in the design of this study.

How were they involved in the recruitment to and conduct of the study?

A: Patients were involved in the conduct of the study by regular follow-up visits and completion of clinical examination and laboratory tests.