Immunosuppressive Therapy in Lupus Nephritis

The Euro-Lupus Nephritis Trial, a Randomized Trial of Low-Dose Versus High-Dose Intravenous Cyclophosphamide

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Objective. **Glomerulonephritis is a severe manifestation of systemic lupus erythematosus (SLE) that is usually treated with an extended course of intravenous (IV) cyclophosphamide (CYC). Given the side effects of this regimen, we evaluated the efficacy and the toxicity of a course of low-dose IV CYC prescribed as a remission-inducing treatment, followed by azathioprine (AZA) as a remission-maintaining treatment.**

Methods. **In this multicenter, prospective clinical trial (the Euro-Lupus Nephritis Trial [ELNT]), we randomly assigned 90 SLE patients with proliferative glomerulonephritis to a high-dose IV CYC regimen (6 monthly pulses and 2 quarterly pulses; doses increased according to the white blood cell count nadir) or a low-dose IV CYC regimen (6 fortnightly pulses at a fixed dose of 500 mg), each of which was followed by AZA. Intent-to-treat analyses were performed.**

Results. **Followup continued for a median of 41.3 months in the low-dose group and 41 months in the high-dose group. Sixteen percent of those in the low-dose group and 20% of those in the high-dose group experienced treatment failure (not statistically significant by Kaplan-Meier analysis). Levels of serum creatinine, albumin, C3, 24-hour urinary protein, and the disease activity scores significantly improved in both groups during the first year of followup. Renal remission was achieved in 71% of the low-dose group and 54% of the high-dose group (not statistically significant). Renal flares were noted in 27% of the low-dose group and 29% of the high-dose group. Although episodes of severe infection were more than twice as frequent in the**

high-dose group, the difference was not statistically significant.

Conclusion. **The data from the ELNT indicate that in European SLE patients with proliferative lupus nephritis, a remission-inducing regimen of low-dose IV CYC (cumulative dose 3 gm) followed by AZA achieves clinical results comparable to those obtained with a high-dose regimen.**

An extended course of high-dose intravenous (IV) cyclophosphamide (CYC), in combination with glucocorticoids, has become the standard treatment of proliferative lupus glomerulonephritis since the pioneering prospective trials performed by the National Institutes of Health (NIH) group that demonstrated the superiority of this regimen over oral (1) or IV (2–4) glucocorticoid therapy alone. Several investigators have, however, raised some concerns about the indiscriminate use of the so-called "NIH regimen" to treat all lupus nephritis patients (5,6). First, the results of the NIH studies, as well as a recent meta-analysis of all randomized trials in lupus nephritis (7), failed to demonstrate that an extended course of IV CYC was superior in terms of renal outcome and survival to other regimens of oral or IV cytotoxic drug(s). Second, high-dose IV CYC treatment is highly toxic; up to 25% of patients develop herpes zoster infection, up to 26% experience a severe infection, and up to 52% of women at risk have ovarian failure (1–4,8). Third, clinically milder cases of biopsy-proven proliferative nephritis—for which less-aggressive treatment might be justified—are now frequently diagnosed because of prompt assessment of early renal involvement.

As an alternative to prolonged intense immunosuppression, we have, over the last 10 years, successfully treated lupus nephritis patients with a sequential regimen consisting of low-dose IV CYC (cumulative dose 3 gm) as a remission-inducing agent, followed by azathioprine (AZA) as a long-term remission-maintaining agent. This approach, supported by retrospective analyses (9–11), has never been validated by a controlled study, however.

Herein we describe the results of the Euro-Lupus Nephritis Trial (ELNT), a European-based multicenter, prospective, randomized study designed to compare high-dose IV CYC (not a strict NIH protocol) and low-dose IV CYC as remission-inducing therapy for proliferative lupus nephritis. AZA was used in both study arms as a long-term immunosuppressive agent to maintain remission.

PATIENTS AND METHODS

Patient selection. Between September 1996 and September 2000, a total of 90 SLE patients were enrolled in the trial at 19 European centers. All patients met the following study criteria: a diagnosis of SLE according to the American College of Rheumatology criteria (12), age ≥14 years, biopsy-proven proliferative lupus glomerulonephritis (World Health Organization [WHO] class III, IV, Vc, or Vd), and proteinuria ≥500 mg in 24 hours. Patients who had taken CYC or AZA during the previous year or had taken ≥15 mg/day of prednisolone (or equivalent) during the previous month were excluded (except for a course of glucocorticoids for a maximum of 10 days before referral). Other exclusion criteria were renal thrombotic microangiopathy, preexisting chronic renal failure, pregnancy, previous malignancy (except skin and cervical intraepithelial neoplasias), diabetes mellitus, previously documented severe toxicity to immunosuppressive drugs, and anticipated poor compliance with the protocol. To avoid selection bias, all nephritis patients who met the inclusion/exclusion criteria were randomized into the trial, except for a few patients who declined to participate.

The study was approved by the ethics committees of all participating hospitals. Written informed consent was obtained from all study patients.

Assessment of renal biopsy samples. Kidney biopsy specimens were assessed (light and immunofluorescence studies) by the renal pathologist at each center. Treatment was decided according to the biopsy classification. Slides of paraffin-embedded sections from all but 1 patient were reviewed by one of us (J-PC), who was blinded to the randomization data. The reviewer evaluated the specimens for activity and chronicity indices, according to the method of Morel-Maroger et al (13).

Briefly, the activity index (maximum score 42) represents the sum of the scores of glomerular hyperactive and active lesions. Hyperactive lesions (hematoxylin bodies, necrosis, circumferential crescents, and necrotizing angiitis) were scored on a scale of 0–6, where 0 = absent, 2 = mild, 4 = moderate, and 6 = severe. Active lesions (endocapillary proliferation, partial crescents, wire-loops, hyalin thrombi, nuclear debris, and acute tubulointerstitial lesions) were scored on a scale of 0–3, where 0 = absent, 1 = mild, 2 = moderate, and 3 = severe. The chronicity index (maximum score 6) was derived by summing the glomerular obsolescence score and the extent of stripes of tubulointerstitial fibrosis. Glomerular obsolescence was scored on a scale of 0–3, where 0 = absent, 1 = 1–29% of the glomeruli, 2 = 30–59% of the glomeruli, and 3 = >60% of the glomeruli. The extent of stripes of tubulointerstitial fibrosis was scored on a scale of 0–3, where 0 = absent, 1 = small, 2 = large, and 3 = diffuse.

Treatment. Immediately after informed consent was obtained and randomization had been performed, all patients received 3 daily pulses of 750 mg of IV methylprednisolone, followed by oral glucocorticoid therapy at an initial dosage of 0.5 mg/kg/day of prednisolone (or equivalent) for 4 weeks. A dosage of 1 mg/kg/day was allowed in critically ill patients (those with renal impairment or severe extrarenal disease). After 4 weeks, glucocorticoid dosages were tapered by 2.5 mg of prednisolone (or equivalent) every 2 weeks. Low-dose

glucocorticoid therapy (5–7.5 mg of prednisolone per day) was maintained at least until month 30 after inclusion.

All patients received IV CYC therapy beginning on day 1 of study inclusion. They were randomized by minimization into 2 treatment groups: high-dose or low-dose IV CYC. With minimization, the group allocation does not rely on chance, but is designed to reduce as much as possible any difference in the distribution of determinants of outcomes (14). The following determinants were taken into account by the minimization: study center, age, sex, history of renal disease, history of glucocorticoid treatment, history of treatment with other immunosuppressive drugs, serum creatinine level, serum albumin level, 24-hour urinary protein level, diastolic blood pressure, European Consensus Lupus Activity Measure (ECLAM) score (15), WHO class, and the presence of crescents, glomerular necrosis, or fibrosis on kidney biopsy specimens.

Patients assigned to the high-dose group received 8 IV CYC pulses within a year (6 monthly pulses followed by 2 quarterly pulses). The initial CYC dose was 0.5 gm/m^2 of body surface area; subsequent doses were increased by 250 mg according to the white blood cell count nadir measured on day 14 (16), with a maximum of 1,500 mg per pulse. Patients assigned to the low-dose group received 6 fortnightly IV CYC pulses at a fixed dose of 500 mg. The use of mesna was left to the decision of the physician. In both treatment arms, AZA (2 mg/kg/day) was started 2 weeks after the last CYC injection and continued at least until month 30 after study inclusion. For cases of AZA-related toxicity, the dosage was reduced to 1 mg/kg/day. Patients who did not tolerate this AZA dosage were dropped from the trial.

Benign renal flares (i.e., those not meeting the definition of severe flares [see below]) were treated with low-dose glucocorticoids (≤15 mg of prednisolone per day) for a 2-week period, hydroxychloroquine (6 mg/kg/day), and/or nonsteroidal antiinflammatory drugs (NSAIDs). A severe renal flare was defined as 1 of the following 3 features: renal impairment, increase in proteinuria, or severe systemic disease. Renal impairment was defined as an SLE-related increase of >33% in the serum creatinine level within a 1-month period. An increase in proteinuria was defined as the recurrence or appearance of nephrotic syndrome (albuminemia <3.5 gm/dl and proteinuria ≥3 gm in a 24-hour sample). In patients with low-grade proteinuria at baseline (≥0.5 gm but <1 gm in 24 hours), a 3-fold increase in 24-hour urinary protein levels within a 3-month period was also considered a severe flare, provided that it was accompanied by microscopic hematuria and a >33% reduction of serum C3 levels within a 3-month period. Severe systemic disease was defined as any of the following events: central nervous system disease, thrombocytopenia (<100,000 platelets/μl), hemolytic anemia, lupus pneumonitis, lupus myocarditis, extensive skin vasculitis, or serositis not responding to low-dose glucocorticoid and/or NSAID treatment. A severe flare was always treated by an increase in the glucocorticoid dosage (0.5–1.0 mg/kg/day of prednisolone) for 1 month, and then promptly tapered to the patient's preflare dosage. Up to 2 IV pulses of methylprednisolone (750 mg) were allowed within a 1-week period.

Hypertension (diastolic blood pressure ≥90 mm Hg) was treated initially with angiotensin-converting enzyme inhibitors, unless contraindicated.

Table 1. Definitions of treatment failure

Treatment failure was defined as either of the following 3 features:

1. Absence of a primary response (applicable only to patients presenting with severe renal disease, which was defined as renal impairment and/or nephrotic syndrome)
   A. For patients with a baseline serum creatinine level ≥1.3 mg/dl but ≤2.6 mg/dl, absence of a primary response was defined as failure of the serum creatinine level to decrease to <1.3 mg/dl at 6 months
   B. For patients with a baseline serum creatinine level >2.6 mg/dl, absence of a primary response was defined as failure of the serum creatinine level to improve by 50% at 6 months
   C. For patients with nephrotic syndrome at baseline (serum albumin level <3.5 gm/dl and 24-hour urinary protein level ≥3 gm/day), but without renal impairment (serum creatinine level <1.3 mg/dl), absence of a primary response was defined as the persistence of nephrotic syndrome at 6 months
2. A glucocorticoid-resistant flare (defined as a severe flare that did not respond to a 1-month increase in the glucocorticoid dosage)
3. A doubling of the serum creatinine level over the lowest value reached at any time during the followup and confirmed on 2 consecutive visits 1 month apart

Contraception was prescribed for all sexually active women of childbearing potential. These patients were also warned against the potential deleterious effects of pregnancy on their disease, at least during the first 30 months after study inclusion.

End points. Patients were evaluated monthly within the first year after study inclusion and quarterly thereafter. Median followup was 41 months (range 8–62 months). In addition to the primary end point, 3 secondary end points were examined.

The primary end point was treatment failure, which was defined as 1 of the following 3 features: absence of a primary response after 6 months of therapy, occurrence of a glucocorticoid-resistant flare, or a doubling of the serum creatinine level. These features are defined in Table 1. Importantly, for patients who presented with impaired renal function, stabilization at that level was considered treatment failure. Patients censored because of treatment failure were treated according to the decisions of their physicians but were included in the intent-to-treat analyses because followup data were available.

The 3 secondary end points were as follows: 1) the kinetics of the response to therapy in the first year, based on serial measurements of serum creatinine, serum albumin, 24-hour urinary protein, and serum C3 levels, as well as the ECLAM score; 2) the rate of renal remission, defined as <10 red blood cells/high-power field and a 24-hour urinary protein level <1 gm, in the absence of a doubling of the serum creatinine level; and 3) the number of severe flares, as defined above.

Statistical analysis. Our estimate of the clinically meaningful difference in the primary outcome was a doubling of the number of patients achieving treatment failure (e.g., a change from 15% to 30%). Given the limited number of patients included in the trial (due to the rarity of the disease and the strict inclusion criteria), the power of our study was low (i.e., 0.22) at the 0.05 significance level.

Table 2. Characteristics of the study subjects at baseline*

	All patients (n = 90)	Patients taking high-dose IV CYC (n = 46)	Patients taking low-dose IV CYC (n = 44)
Age, years			
Mean ± SD	31 ± 11	30 ± 11	33 ± 12
Range	14–72	14–72	14–62
No. of females/males	84/6	43/3	41/3
Race, no. of patients			
Caucasian	76	37	39
Asian	6	4	2
African Caribbean/black	8	5	3
History, no. of patients			
Renal disease	21	11	10
Glucocorticoid therapy	55	27	28
Other immunosuppressive therapy	7	4	3
ECLAM score			
Mean ± SD	6.8 ± 2.0	6.6 ± 1.8	7.0 ± 2.2
Range	3–10	3–10	3–10
Diastolic BP ≥90 mm Hg, no. of patients	42	22	20
Serum creatinine, mg/dl			
Mean ± SD	1.15 ± 0.66	1.21 ± 0.76	1.09 ± 0.54
Range	0.5–4.8	0.6–4.8	0.5–3.2
Serum albumin, gm/dl			
Mean ± SD	3.03 ± 0.61	2.96 ± 0.62	3.01 ± 0.60
Range	1.6–4.6	2.0–4.5	1.6–4.6
24-hour urinary protein, gm			
Mean ± SD	3.04 ± 2.39	3.17 ± 2.43	2.90 ± 2.37
Range	0.5–12.2	0.5–12.2	0.5–11.6
WHO class nephritis, no. of patients			
Class III	21	10	11
Class IV	62	31	31
Class Vc/Vd	7	5	2
Renal biopsy activity index†			
Mean ± SD	9.9 ± 6.1	10.7 ± 6.6‡	9.1 ± 5.7
Range	1–26	1–26	2–19
Renal biopsy chronicity index§			
Mean ± SD	0.8 ± 0.8	0.8 ± 0.8‡	0.8 ± 0.9
Range	0–3	0–3	0–3
Glomerular crescents, no. of patients			
Partial	42	22‡	20
Circumferential	12	5‡	7
Glomerular necrosis, no. of patients	16	8‡	8

* Statistical analysis of between-group differences was not performed on the parameters taken into account by the minimization procedure (see Patients and Methods). For the other parameters, all *P* values were greater than 0.05. IV = intravenous; CYC = cyclophosphamide; ECLAM = European Consensus Lupus Activity Measure; BP = blood pressure; WHO = World Health Organization.
† Maximum possible score 42, according to the criteria of Morel-Maroger et al (13).
‡ Data were available for only 42 patients.
§ Maximum possible score 6, according to the criteria of Morel-Maroger et al (13).

Survival curves were derived using the Kaplan-Meier method and were statistically tested with the log rank test. We calculated the hazard ratios (HRs) and their 95% confidence intervals (95% CIs) using the univariate Cox proportional hazards model. Patients dropped from the trial were included in all Kaplan-Meier analyses (intent-to-treat analyses). Serial data were compared within and between groups by repeated-measures analysis of variance, with a "between groups" and a "repeated measures" comparison. Unpaired *t*-tests or Mann-Whitney U tests were used for between-group comparisons, as appropriate.
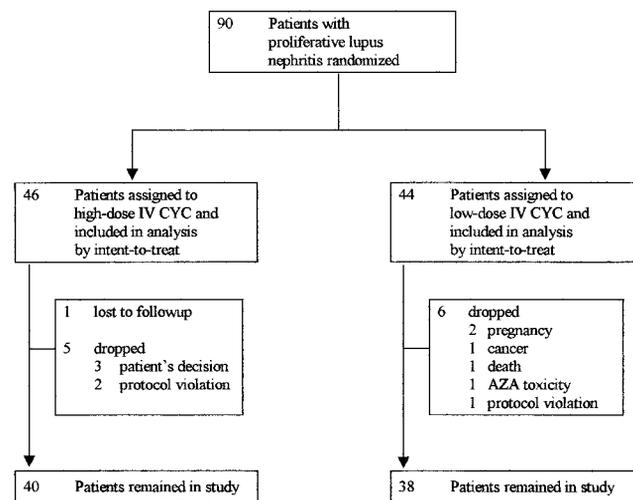
RESULTS

Baseline data and treatment. Forty-six patients were assigned to the high-dose IV CYC group and 44 to the low-dose IV CYC group. Their baseline clinical, biochemical, and kidney pathology data are detailed in Table 2. Of the 90 patients, 20 (22%) presented with renal impairment (serum creatinine level ≥1.3 mg/dl)
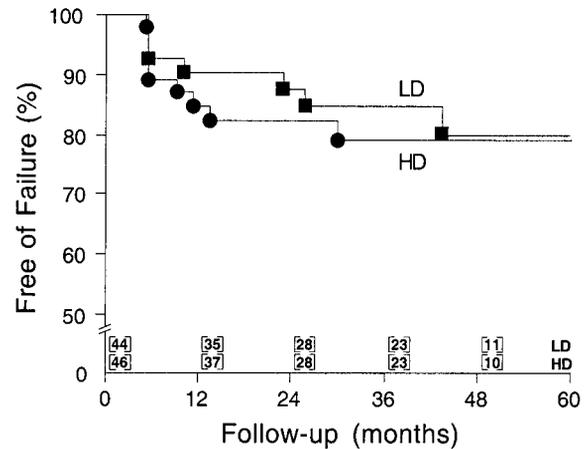
and 25 (28%) presented with nephrosis (24-hour urinary protein ≥3.5 gm). Of note, this was the first episode of nephritis in 67 patients (74%). Only 8% of the patients had previously been treated with immunosuppressive drugs other than glucocorticoids.

Mean (±SD) daily starting doses of prednisolone were similar in the 2 groups (33 ± 11 mg/day in the high-dose group versus 36 ± 13 mg/day in the low-dose group; $P = 0.23$). The mean (±SD) cumulative dose of IV CYC prescribed for patients in the high-dose group (before dropout or treatment failure) was 8.5 ± 1.9 gm. All patients who were randomized into the low-dose group received a cumulative IV CYC dose of 3 gm (before dropout or treatment failure), except for 1 patient who died on day 28 (see below). Cumulatively, angiotensin-converting enzyme inhibitors were used by 52% and 59% of patients in the high-dose and low-dose groups, respectively.

As indicated in Figure 1, 1 patient (assigned to the high-dose treatment) was lost to followup at week 44, and 11 patients (12%) were dropped from the trial for the following reasons: patient's decision (n = 3), protocol violation (n = 3), death (n = 1), pregnancy (n = 2), cancer (n = 1), and AZA toxicity (n = 1). After being dropped from the study, patients were treated according to the decisions of their physicians. Followup was available for all of them, thereby allowing analyses by intent-to-treat.

Figure 2. Kaplan-Meier analysis of the probability of an absence of treatment failure. Patients were randomized to a low-dose (LD; ■) or a high-dose (HD; ●) regimen of intravenous cyclophosphamide, followed by azathioprine treatment. Treatment failure was defined as 1 of the following 3 features: absence of a primary response after 6 months of therapy, occurrence of a glucocorticoid-resistant flare, or a doubling of the serum creatinine level (features are defined in Table 1). The hazard ratio for treatment failure in the low-dose group compared with the high-dose group was 0.79 (95% confidence interval 0.30–2.14; $P = 0.64$). Numbers shown along the abscissa are the number of patients at risk in each group. Analysis was by intent-to-treat.


Figure 1. Trial profile, showing the number of patients enrolled, the number of patients assigned to high-dose and low-dose intravenous (IV) cyclophosphamide (CYC), the number of dropouts from each group, and the number of patients who remained in the study. AZA = azathioprine.

Primary end point. After a median followup of 41.3 months for patients in the low-dose group and 41 months for those in the high-dose group, 7 of the 44 low-dose patients (16%) and 9 of the 45 high-dose patients (20%) experienced treatment failure, which was the primary end point of the study. The Kaplan-Meier curves shown in Figure 2 indicate that there was no significantly greater cumulative probability of treatment failure in patients given a low-dose IV CYC regimen than in those given a high-dose IV CYC regimen (HR 0.79, 95% CI 0.30–2.14; $P = 0.64$). The reason for failure was as follows: absence of a primary response in 2 of the low-dose and 4 of the high-dose patients, a glucocorticoid-resistant flare in 2 low-dose and 2 high-dose patients, and doubling of the serum creatinine level in 3 low-dose and 3 high-dose patients.

The baseline clinical, biochemical, and pathologic data for the 16 patients who experienced treatment failure did not differ from the data in the patients who did not experience treatment failure (data not shown). Treatment failure was not significantly more frequent in patients with WHO class IV nephritis compared with patients with WHO class III nephritis, nor in nonwhite patients compared with white patients (data not shown).
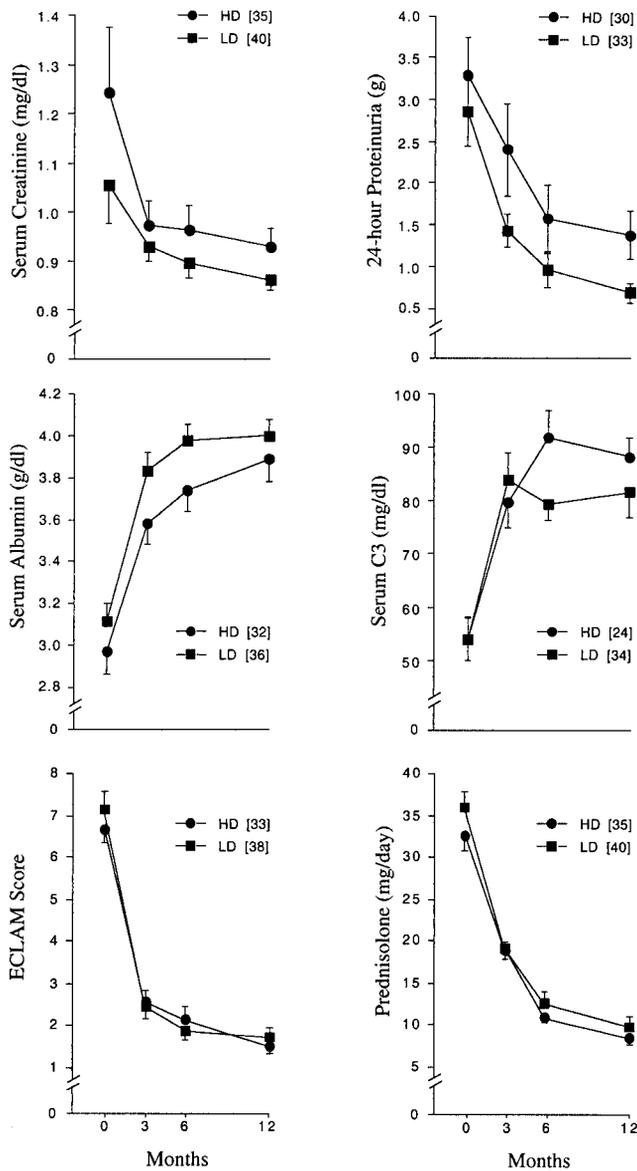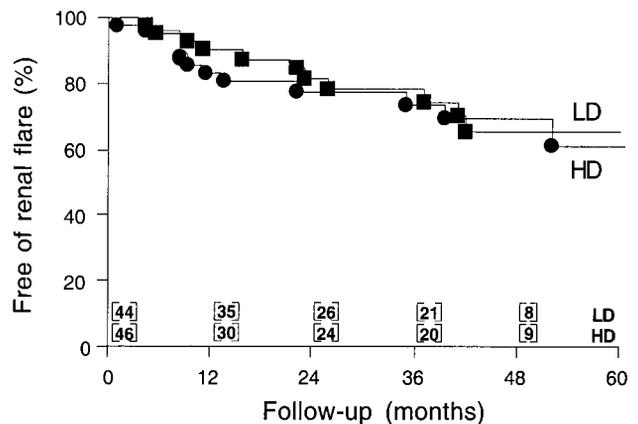
Figure 3. Kinetics of the initial response to therapy. Patients were randomized to a low-dose (LD) or a high-dose (HD) regimen of intravenous cyclophosphamide, followed by azathioprine treatment. Values are the mean ± SEM. Repeated-measures analysis of variance yielded $P < 0.005$ for all "repeated measures" analyses and $P > 0.05$ for all "between groups" comparisons. Numbers in parentheses are the number of patients considered in this analysis (with data available at every time point). Analysis was by intent-to-treat. ECLAM = European Consensus Lupus Activity Measure.

Secondary end points. Figure 3 shows the kinetics of the initial response to therapy. Serum creatinine, serum albumin, 24-hour urinary protein, serum C3 titers, and the ECLAM score significantly improved in
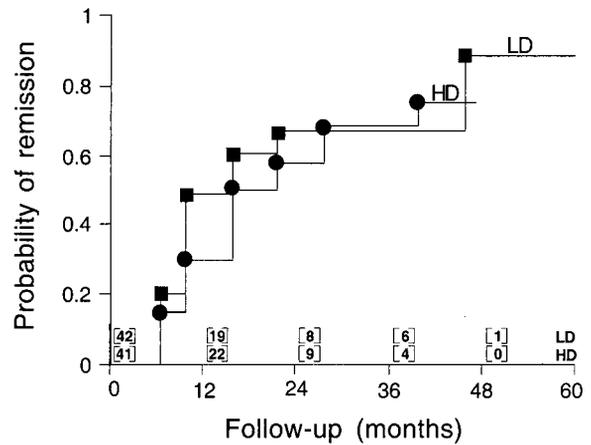
Figure 4. Kaplan-Meier analysis of the probability of renal remission. Patients were randomized to a low-dose (LD; ■) or a high-dose (HD; ●) regimen of intravenous cyclophosphamide, followed by azathioprine treatment. Renal remission was defined as <10 red blood cells/high-power field and a 24-hour urinary protein level <1 gm, in the absence of a doubling of the serum creatinine level. The hazard ratio for renal remission in the low-dose group compared with the high-dose group was 1.26 (95% confidence interval 0.72–2.21; $P = 0.36$). Numbers shown along the abscissa are the number of patients at risk in each group. Analysis was by intent-to-treat.

both groups during the first year of followup ($P \leq 0.005$). No significant difference was noted between patients in the low-dose and high-dose IV CYC groups for any of the parameters examined ($P > 0.05$). Impor-

Figure 5. Kaplan-Meier analysis of the probability of renal flare. Patients were randomized to a low-dose (LD; ■) or a high-dose (HD; ●) regimen of intravenous cyclophosphamide, followed by azathioprine treatment. The hazard ratio for renal flare in the low-dose group compared with the high-dose group was 0.90 (95% confidence interval 0.40–2.04; $P = 0.80$). Numbers shown along the abscissa are the number of patients at risk in each group. Analysis was by intent-to-treat.

Table 3. Adverse events*

Adverse event	All patients (n = 89)	Patients taking high-dose IV CYC (n = 45)	Patients taking low-dose IV CYC (n = 44)
Death	2	0	2
End-stage renal disease	3	2	1
Doubling of serum creatinine level	4	1	3
Severe infection	15	10	5
Total no. of episodes	24	17	7
Type of severe infection			
Pneumonia	7	4	3
Other bacterial infection	6	5	1
Cytomegalovirus	4	3	1
Herpes zoster	7	5	2
Other infection	12	7	5
Total no. of episodes	20	10	10
Type of other infection			
Mucocutaneous	9	5	4
Lower urinary tract	7	2	5
Upper respiratory tract	3	2	1
Ear, nose, and throat	1	1	0
Hematologic toxicity			
Leukopenia (≤4,000/μl)	10	5†	5†
Toxic anemia	1	0	1‡
Bone marrow aplasia	1	0	1‡
Gonadal toxicity			
Menopause	4	2§	2¶
Transient amenorrhea	2	1	1
Other adverse events			
Azathioprine-induced hepatitis	3	0	3
Ischemic heart disease	3	1	2
Deep vein thrombosis	2	2	0
Diabetes	2	1	1
Avascular osteonecrosis	1	1	0
Tendon rupture	1	0	1

* Because of the small numbers of events, Kaplan-Meier analyses were not performed on these data, except for the cumulative probability of severe infection (see Figure 6). Values are the number of patients, except for the values for the number of episodes and types of both severe infection (those requiring in-patient antimicrobial therapy) and other infection. IV = intravenous; CYC = cyclophosphamide.
† During pulse CYC therapy in 2 patients.
‡ While taking azathioprine.
§ At age 20 years and age 55 years, respectively.
¶ Both at age 44 years.

tantly, the glucocorticoid-tapering regimens did not differ between groups as shown in Figure 3 ($P > 0.05$). A subset analysis performed on patients presenting with renal impairment (serum creatinine ≥1.3 mg/dl) revealed a similar response to the 2 treatments (data not shown).

Achievement of renal remission at any time during followup could be evaluated in 83 patients for whom regular quarterly followup data were available. Thirty of the 42 evaluable patients in the low-dose group (71%) and 22 of the 41 evaluable patients in the high-dose group (54%) achieved renal remission. The Kaplan-Meier curves shown in Figure 4 indicate that the cumulative probability of achieving renal remission did not

differ between patients given a low-dose regimen versus those given a high-dose regimen (HR 1.26, 95% CI 0.72–2.21; $P = 0.36$).

Twelve of the 44 patients in the low-dose group (27%) and 13 of the 45 patients in the high-dose group (29%) experienced renal flare. Again, Kaplan-Meier analysis failed to detect a significant difference in the cumulative probability of renal flare between patients given a low-dose regimen versus those given a high-dose regimen (HR 0.90, 95% CI 0.40–2.04; $P = 0.80$) (Figure 5). Six of the 25 patients with renal flare had a doubling of the serum creatinine level at the last followup visit; 2 of these 6 patients had end-stage renal disease (ESRD). Four additional patients (1 low-dose and 3 high-dose)

experienced a severe extrarenal flare. Of note, of the 16 patients in the high-dose group who experienced a renal flare, 7 experienced the flare while being treated with CYC pulses, whereas the remaining 9 patients experienced the flare while taking AZA. As anticipated, based on the duration of CYC pulse therapy in the low-dose group, all but 1 of the 13 patients in this group who experienced a flare did so while taking AZA.

Adverse events. All adverse events occurring between study inclusion and the last followup visit were recorded (Table 3). Two patients, both in the low-dose group, died. One of the patients (age 14 years) presented with renal impairment, nephrotic syndrome, and congestive heart failure. Despite treatment with IV methylprednisolone and CYC (500 mg on day 1 and day 14), she developed multiorgan failure on day 20, and on day 28, she died. The other patient (age 51 years), was dropped from the trial during week 121 because of breast cancer. This patient died suddenly of unknown causes during week 194.

Three patients, 2 in the high-dose group and 1 in the low-dose group, progressed to ESRD (at week 104, 193, and 208, respectively) and are currently undergoing dialysis. Two of them had been censored because of the absence of a primary response; the third patient had been censored because of doubling of the serum creatinine level. In addition to these 3 patients who reached ESRD, 4 others (1 in the high-dose and 3 in the low-dose group) experienced a doubling of their serum creatinine level by the time of the last visit. Three of them had been censored because of doubling of the serum creatinine level, and the fourth had been dropped because of pregnancy.

Severe infections (those requiring inpatient antimicrobial therapy) were noted in 10 patients from the high-dose group (17 episodes) and in 5 patients from the low-dose group (7 episodes). As indicated in Figure 6, the cumulative probability of severe infection was similar in both groups (HR 0.50, 95% CI 0.17–1.47; $P = 0.20$), as was the number of severe infections/patient-year (mean $\pm$ SD 0.14 $\pm$ 0.36 in the high-dose group versus 0.06 $\pm$ 0.22 in the low-dose group; $P = 0.35$).

As indicated in Table 3, similar numbers of patients in both groups experienced hematologic and gonadal toxicity (defined clinically in premenopausal women as a prolonged absence of menstrual periods). Only 1 patient (age 20 years) developed premature ovarian failure. This patient was in the high-dose group and received oral CYC after treatment failure at week 49. The other 3 patients were all over the age of 40 years when they became menopausal.
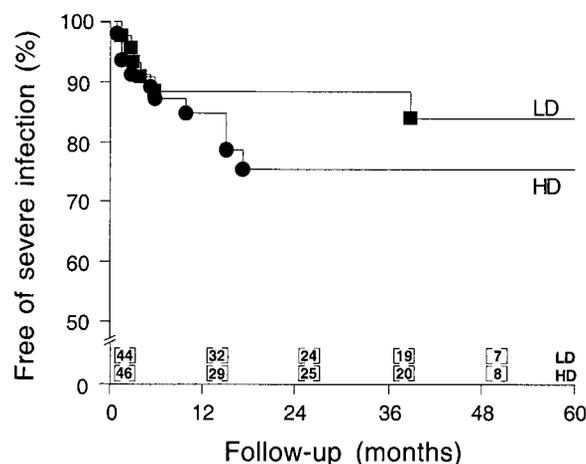
Figure 6. Kaplan-Meier analysis of the probability of absence of severe infection. Patients were randomized to a low-dose (LD; ■) or a high-dose (HD; ●) regimen of intravenous cyclophosphamide, followed by azathioprine treatment. The hazard ratio for severe infection in the low-dose group compared with the high-dose group was 0.50 (95% confidence interval 0.17–1.47; $P = 0.20$). Numbers shown along the abscissa are the number of patients at risk in each group. Analysis was by intent-to-treat.

Outcome after treatment failure. Table 4 summarizes the immunosuppressive treatment(s) prescribed to patients after failure of the study treatment. Eight (5 in the high-dose and 3 in the low-dose group) of the 16 patients censored because of treatment failure had normal renal function by the time of the last followup visit. Most of the other 8 patients who did not recover normal kidney function had been censored, as expected, because of doubling of the serum creatinine level.

DISCUSSION

The ELNT is a multicenter, prospective, randomized study designed to test whether a low-dose IV CYC regimen (6 fortnightly pulses of 500 mg; cumulative dose 3 gm), followed by AZA, is an effective therapy for proliferative lupus glomerulonephritis, as suggested by retrospective analyses (9–11). The low-dose IV CYC regimen was compared with a high-dose IV CYC treatment (6 monthly pulses and 2 quarterly pulses, with doses increased according to the white blood cell count nadir). In both treatment arms, AZA was used as long-term immunosuppressive therapy. The results of the trial indicate that 1) there was no significantly greater cumulative probability of treatment failure in patients taking a low-dose IV CYC regimen than in those taking a high-dose regimen, 2) the kinetics of the

Table 4. Outcome after treatment failure

			Outcome	
Group, patient	Reason for treatment failure*	Immunosuppressive treatment after failure†	Kidney function‡	24-hour urinary protein ≥3.5 gm§
Low-dose IV CYC				
0101	GCRF	IV CYC	Normal	–
0201	APR	IV CYC, 6-MP, MMF	ESRD	NA
1002	DSC	IV CYC	DSC	–
1201	DSC	IV CYC, CSA	Impaired	–
1202	DSC	CSA	DSC	–
1205	APR	CSA	Normal	–
2601	GCRF	IV CYC, MMF, CSA	Normal	–
High-dose IV CYC				
0204	APR	IV CYC	Normal	+
0206	GCRF	IV CYC, MMF, CSA	Normal	–
0208	DSC	IV CYC	Normal	–
0218	APR	MMF	Impaired	+
1103	GCRF	CSA	Normal	+
1802	DSC	IV CYC	ESRD	NA
1806	APR	CSA	ESRD	NA
1811	APR	IV CYC	Normal	–
2909	DSC	Oral CYC, MMF	DSC	+

* GCRF = glucocorticoid-resistant flare; APR = absence of primary response; DSC = doubling of the serum creatinine level.
† IV = intravenous; CYC = cyclophosphamide; 6-MP = 6-mercaptopurine; MMF = mycophenolate mofetil; CSA = cyclosporin A.
‡ Impaired kidney function was defined as a serum creatinine level ≥1.3 mg/dl, without doubling of the serum creatinine level. ESRD = end-stage renal disease; DSC = doubling of the serum creatinine level.
§ NA = not applicable.

initial response did not differ between the two groups, 3) the cumulative probability of achieving renal remission was similar in both groups, and 4) the number of renal flares did not differ between the two groups. Severe infectious side effects were less common in the low-dose group, although the difference was not statistically significant.

These data suggest that good clinical results may be achieved even with a low cumulative dose of 3 gm of IV CYC. Our findings therefore call into question the current practice, based on the NIH trials, of treating all lupus nephritis patients with an extended course of IV CYC.

There are, however, differences between the ELNT and the NIH studies. First, most patients included in the ELNT did not have clinically severe kidney disease. Although all patients had proliferative glomerulonephritis, only 22% presented with renal impairment and 28% presented with nephrosis, compared with 64% and 62% respectively, in the study by Boumpas et al (2). It should be stressed, however, that the patients randomized into the ELNT are, by definition, representative of those currently treated in our lupus clinics. Milder cases of proliferative lupus nephritis, for which less-aggressive

treatment is certainly justified, are now frequently diagnosed due to the prompt assessment of kidney involvement, particularly in lupus patients presenting without renal signs at diagnosis and in whom proteinuria is detected and investigated as early as possible through regular followup visits.

Second, few black or African Caribbean patients were included in the ELNT (9% of the cohort); this is in contrast to the high percentage of black patients randomized into the NIH studies reported by Boumpas et al (43%) (2) and Gourley et al (34%) (3). Since the outcome of lupus nephritis is poorer in black patients compared with the outcome in white patients (17), the underrepresentation of this ethnic group in our European populations might explain, at least in part, why a low-dose IV CYC regimen was effective in most of our patients.

Third, the duration of the high-dose IV CYC regimen prescribed in the ELNT is shorter than that of the studies conducted in North America, and as a consequence, the cumulative dose of IV CYC is lower. Given the high incidence of gonadal failure (38–52% of women at risk) associated with the standard NIH regi-

men, we decided to reduce the number of pulses in the high-dose regimen, using 8 instead of 14. As a consequence, comparison with trials in which a more intense and prolonged high-dose regimen was prescribed might be difficult.

Finally, the use of AZA for long-term immunosuppression after completion of the CYC pulses is yet another difference between the ELNT and the previously published trials using IV CYC. In the ELNT regimen, a potentially toxic drug is prescribed for a short period of time as remission-inducing therapy and a possibly less-toxic drug is prescribed for a longer period of time as remission-maintaining treatment. By securing long-term immunosuppression, this sequential treatment might have contributed to the good results achieved in patients given a low-dose regimen of IV CYC. It should be stressed, however, that a significant number of patients in each group (9 in the high-dose and 12 in the low-dose group) experienced at least 1 flare, mostly renal, while they were being treated with AZA. Other remission-maintaining drugs should therefore be tested in lupus nephritis. The newly developed immunosuppressive drug mycophenolate mofetil, an uncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase (18), is promising in this respect given its superiority over AZA in reducing the incidence of episodes of acute rejection after renal and cardiac transplantation (19,20) and its successful short-term use, together with high-dose glucocorticoid, in Chinese patients with SLE and proliferative glomerulonephritis (21).

Despite the aforementioned differences between the designs of the ELNT and the NIH trials, the results presented here will be compared with those of a strict NIH protocol in terms of renal remission, renal relapse, and poor renal outcome. The rate of renal remission (71% in the low-dose group) is similar to that observed with the classic NIH regimen (62%) in a group of patients with comparable baseline disease severity (3). The rate of renal relapse in our patients is higher than that reported in patients given a classic NIH protocol (7% reported by Gourley et al [3]). It should be stressed, however, that we took into account all renal flares, whereas only those occurring after completion of the monthly phase of treatment (2) or only those occurring after achieving remission and maintaining it for 1 year (3) were counted in the two recent NIH trials. More importantly, a possible difference in renal relapse rates should be weighted against the very high risk (up to 52%) of premature ovarian failure associated with the

classic NIH regimen (1–4,8), a side effect explaining why a significant proportion of patients now decline high-dose IV CYC therapy. Finally, the rates of poor renal outcome are comparable. Thus, the percentage of patients whose serum creatinine level eventually doubled was 7% in our high-dose group and 9% in our low-dose group, while this value varied between 4% and 24% in the recent NIH trials (2–4).

The incidence of major side effects did not differ significantly between the high-dose and the low-dose groups, probably because of the relatively low numbers of patients included in the study. The number of patients with severe infections and the number of episodes of severe infection were, however, at least twice as high in patients taking the high-dose treatment. As indicated by the Kaplan-Meier analysis shown in Figure 6, the cumulative probability of severe infection increased maximally in both groups within the first months of treatment, stabilizing after 6 months in the low-dose group. In contrast, in the high-dose group, additional patients experienced their first severe infectious event after 6 months of treatment. The possibility that the cumulative probability of severe infection would have been greater had a standard NIH regimen been applied would be consistent with published data (1–4).

Only a very few patients experienced permanent ovarian failure, a not-unexpected finding given the cumulative dose of IV CYC prescribed, even to high-dose patients (8). Whether or not women given high-dose IV CYC therapy will reach menopause earlier than those given low-dose therapy, will be addressed in an analysis after longer followup.

The issue of whether a 500-mg pulse of CYC could be given orally rather than intravenously was not addressed in this trial. Although the bioavailability of oral CYC is excellent (22), gastrointestinal side effects, such as nausea and vomiting, might compromise optimum compliance and introduce confusion about the amount of CYC that is actually being absorbed (23).

Although caution should be exercised in extrapolating the results of the ELNT to other lupus nephritis populations with different ethnic backgrounds or disease severity, the many advantages of the ELNT regimen should be emphasized. A 500-mg pulse of CYC can be administered, with excellent immediate tolerance, on an outpatient basis as a 30-minute infusion, without the need for IV antiemetics and forced hydration. The costs of therapy and, possibly, the cumulative dose-dependent long-term toxicity would thereby be reduced.

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