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Sequential Therapies for Proliferative Lupus Nephritis

Gabriel Contreras, M.D., M.P.H., Victoriano Pardo, M.D., Baudouin Leclercq, M.D., Oliver Lenz, M.D., Elaine Tozman, M.D., Patricia O’Nan, R.N., and David Roth, M.D.

ABSTRACT

BACKGROUND

Long-term therapy with cyclophosphamide enhances renal survival in patients with proliferative lupus nephritis; however, the beneficial effect of cyclophosphamide must be weighed against its considerable toxic effects.

METHODS

Fifty-nine patients with lupus nephritis (12 in World Health Organization class III, 46 in class IV, and 1 in class Vb) received induction therapy consisting of a maximum of seven monthly boluses of intravenous cyclophosphamide (0.5 to 1.0 g per square meter of body-surface area) plus corticosteroids. Subsequently, the patients were randomly assigned to one of three maintenance therapies: quarterly intravenous injections of cyclophosphamide, oral azathioprine (1 to 3 mg per kilogram of body weight per day), or oral mycophenolate mofetil (500 to 3000 mg per day) for one to three years. The base-line characteristics of the three groups were similar, with the exception that the chronicity index was 1.9 points lower in the cyclophosphamide group than in the mycophenolate mofetil group ($P=0.009$).

RESULTS

During maintenance therapy, five patients died (four in the cyclophosphamide group and one in the mycophenolate mofetil group), and chronic renal failure developed in five (three in the cyclophosphamide group and one each in the azathioprine and mycophenolate mofetil groups). The 72-month event-free survival rate for the composite end point of death or chronic renal failure was higher in the mycophenolate mofetil and azathioprine groups than in the cyclophosphamide group ($P=0.05$ and $P=0.009$, respectively). The rate of relapse-free survival was higher in the mycophenolate mofetil group than in the cyclophosphamide group ($P=0.02$). The incidence of hospitalization, amenorrhea, infections, nausea, and vomiting was significantly lower in the mycophenolate mofetil and azathioprine groups than in the cyclophosphamide group.

CONCLUSIONS

For patients with proliferative lupus nephritis, short-term therapy with intravenous cyclophosphamide followed by maintenance therapy with mycophenolate mofetil or azathioprine appears to be more efficacious and safer than long-term therapy with intravenous cyclophosphamide.

From the Dialysis Unit (G.C.) and the Electron Microscopy Unit (V.P.), Veterans Affairs Medical Center and University of Miami; and the Divisions of Nephrology (B.L., O.L., P.O., D.R.) and Rheumatology and Immunology (E.T.), University of Miami — both in Miami. Address reprint requests to Dr. Contreras at the Division of Nephrology, 1600 NW 10th Ave. R-126, Miami, FL 33136, or at gcontrer@med.miami.edu.

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LUPUS NEPHRITIS INCREASES THE MORBIDITY and mortality associated with systemic lupus erythematosus. The long-term follow-up of affected patients has demonstrated that focal and diffuse proliferative forms of glomerulonephritis (World Health Organization [WHO] classes III and IV, respectively) progress to chronic renal failure in the absence of appropriate immunosuppressive treatment.¹ Long-term therapy with cyclophosphamide enhances renal survival in patients with proliferative forms of lupus nephritis but has considerable adverse effects.²⁻⁷ Chan et al. demonstrated that in such patients, short-term therapy with oral cyclophosphamide and corticosteroids can induce remission and can safely be changed to long-term therapy with oral azathioprine and corticosteroids without a substantial risk of progression to chronic renal failure and toxic effects.^{8,9}

The efficacy and safety of mycophenolate mofetil, which inhibits purine synthesis and has antiproliferative effects on lymphocytes and profoundly attenuates the production of autoantibodies by B cells,¹⁰ have been demonstrated in a rodent model of lupus nephritis¹¹ and in patients with diffuse proliferative lupus nephritis.⁹ We compared the efficacy and safety of two sequential regimens — intravenous cyclophosphamide followed by either oral mycophenolate mofetil or azathioprine — with the efficacy and safety of long-term therapy with intravenous cyclophosphamide.

METHODS

STUDY DESIGN AND STUDY POPULATION

We conducted a single-center, randomized, open-label, controlled trial between August 1996 and May 2003. Patients meeting the diagnostic criteria for systemic lupus erythematosus according to the American Rheumatism Association¹² who had undergone a kidney biopsy were screened to identify those who were 18 years of age or older and who had received a histologic diagnosis of proliferative lupus nephritis (WHO class III, IV, or Vb). Exclusion criteria were a creatinine clearance that was consistently less than 20 ml per minute, any clinically significant infection, pregnancy, the receipt of more than seven doses of intravenous cyclophosphamide, or the receipt of azathioprine for longer than eight weeks. The institutional review board on human research approved the study, and written informed consent was obtained from all patients.

Renal-biopsy specimens were examined by light,

immunofluorescence, and electron microscopy and were categorized according to the WHO classification¹³ by two renal pathologists who were unaware of the patient's treatment assignment. One pathologist estimated the activity and chronicity indexes according to the scoring system of Pollak et al.,¹⁴ as modified by Austin et al.¹⁵

IMMUNOSUPPRESSIVE PROTOCOLS

Induction therapy consisted of a maximum of seven monthly boluses of intravenous cyclophosphamide (0.5 to 1.0 g per square meter of body-surface area, to induce a nadir leukocyte count that was no lower than 2000 cells per cubic millimeter) and corticosteroids. After induction, the patients were randomly assigned, in order of enrollment by means of sealed envelopes (stratified in two groups: blacks and other patients), to one of three regimens of maintenance therapy: 0.5 to 1.0 g of intravenous cyclophosphamide (Cytoxan, Bristol-Myers Squibb) per square meter every three months, 1 to 3 mg of oral azathioprine per kilogram of body weight per day, and 500 to 3000 mg of oral mycophenolate mofetil (CellCept, Roche) per day, with the dose titrated to minimize gastrointestinal side effects and to maintain a leukocyte count of no less than 2000 cells per cubic millimeter. All patients receiving cyclophosphamide also received mesna (Mesnex, Bristol-Myers Squibb) to prevent hemorrhagic cystitis and granisetron hydrochloride (Kytril, Glaxo-SmithKline) to prevent nausea and vomiting.

All three groups received maintenance immunosuppressive therapy with oral prednisone (up to 0.5 mg per kilogram per day) or an equivalent corticosteroid for one to three years. Maintenance immunosuppressive therapy was stopped in the event of persistent leukopenia (a white-cell count below 2000 per cubic millimeter), pregnancy, severe gastrointestinal upset, persistent elevation of liver-enzyme levels to more than three times the upper limit of the normal range, severe sepsis, or the patient's refusal to continue.

The following studies were performed monthly during induction therapy and quarterly during maintenance therapy: measurement of serum creatinine, blood urea nitrogen, albumin, and liver-enzyme levels; a complete blood count 7 to 14 days after a cyclophosphamide pulse; urinalysis; measurement of protein and creatinine levels in 24-hour urine collections or, at random times, calculation of the ratio of protein to creatinine in urine; and measurement of antinuclear antibodies (ANA), anti-

bodies against double-stranded DNA, complement C3 and C4, and anticardiolipin antibodies (when appropriate). The patients were seen monthly during induction therapy and quarterly during maintenance therapy, and drug-related adverse effects and end points were tabulated.

END POINTS AND DEFINITIONS

The primary end points of the study were patient and renal survival. Chronic renal failure was defined as a sustained increase (for more than four months) in the serum creatinine value to at least twice the lowest value reached during the induction phase of the study or the need for long-term maintenance dialysis or transplantation. The secondary end points of the study were renal relapse, as defined by a doubling of the urinary protein:creatinine ratio (proteinuric) or by an increase in the serum creatinine level of 50 percent or more for more than 1 month (nephritic); amenorrhea for 12 months or more (no prophylactic hormonal therapy was used to diminish the risk of ovarian damage); and hospitalization, infection, and other adverse events. Remission was defined as a decrease in the urinary protein:creatinine ratio to less than 3 in patients with base-line proteinuria in the nephrotic range (a urinary protein:creatinine ratio of at least 3) or by 50 percent in patients with subnephrotic proteinuria accompanied by either an improvement in the base-line serum creatinine level of at least 25 percent or a stable serum creatinine level that was within 25 percent of the base-line level.

STATISTICAL ANALYSIS

The principal analysis was performed with the use of survival statistics. The cumulative survival curves were derived by means of the Kaplan–Meier method, and the differences between survival curves were compared by means of the log-rank test.^{16,17} In the analysis of renal survival, data were censored at the time of loss to follow-up or death. In the analyses of patient survival and the composite end point of death or chronic renal failure, data were censored at the time of loss to follow-up. Assuming a 90 percent rate of renal survival as a reference value in the cyclophosphamide group and a rate of 65 percent in the azathioprine and mycophenolate mofetil groups, 20 patients were required in each group for the study to achieve a power of 80 percent at a significance level of 0.05 to detect an absolute difference of 25 percent between pairs of groups in the development of chronic renal failure during a

5.5-year period, after allowance for a 5 percent loss to follow-up.

The relapse-free survival was calculated according to the Kaplan–Meier method, and the differences between each pair of survival curves was analyzed by means of the log-rank test. The rate of sustained amenorrhea was calculated in each maintenance-therapy group as the incidence density: $100 \times (\text{the number of patients} \div \text{the number of patient-years of follow-up})$. The rates were compared between groups by means of a normal distribution approximation.¹⁸ Only women with regular menses who were 18 to 50 years of age at entry were included in this analysis. Similarly, infection rates were calculated and compared between groups. The differences between groups in the likelihood of not requiring hospitalization were compared by means of the log-rank test. Completion of six months of maintenance therapy was considered to be adequate exposure to the intervention; however, no patients were excluded from analyses if they did not meet this criterion.

At the beginning of the induction and the maintenance phases, we compared categorical and continuous variables among the three groups using χ^2 tests or analysis of variance as appropriate. Data are presented as means \pm SD. All reported P values are based on two-tailed tests without adjustment for multiple comparisons. All statistical analyses were performed with the use of the NCSS 2000 software package.

The trial was designed by the investigators. The investigators also managed the data base and data accrual, analyzed the data, and interpreted the results.

RESULTS

CHARACTERISTICS OF THE PATIENTS

A total of 113 patients with systemic lupus erythematosus underwent a kidney biopsy between May 1995 and July 2002. Fifty-three met the exclusion criteria, and thus 60 patients underwent randomization. One patient assigned to the azathioprine group was subsequently excluded after a history of therapy with oral azathioprine for longer than eight weeks was confirmed. As compared with the randomized patients, the excluded patients were less likely to have WHO class III or IV nephritis ($P < 0.001$), had a lower activity-index score ($P < 0.001$), and had a higher ANA titer ($P = 0.02$).

At the beginning of induction therapy, the char-

acteristics of the patients in the three groups were similar, with the exception that the chronicity index in the cyclophosphamide group was 1.9 points lower than that in the mycophenolate mofetil group ($P=0.009$). At the beginning of the maintenance therapy, the three groups also had similar characteristics, with the exception that the ANA titer was lower in the cyclophosphamide group than in the azathioprine group ($P=0.04$) (Table 1).

IMMUNOSUPPRESSIVE TREATMENT

The median duration of treatment was 25 months in the cyclophosphamide group, 29 months in the mycophenolate mofetil group, and 30 months in the azathioprine group ($P=0.38$). The shorter median length of treatment in the cyclophosphamide group was associated with more deaths and events related to chronic renal failure that led to the termination of treatment.

During induction therapy, treatment was similar among the three groups. The mean dose of intravenous cyclophosphamide was 541 ± 40 mg per square meter. Forty-two patients received six pulses of monthly intravenous cyclophosphamide (13 in the cyclophosphamide group, 13 in the mycophenolate mofetil group, and 16 in the azathioprine group), 8 received five pulses (1, 5, and 2, respectively), 6 received seven pulses (4, 1, and 1, respectively), and 3 received four pulses (2 in the cyclophosphamide group and 1 in the mycophenolate mofetil group) ($P=0.20$ for the total chi-square). The patients who had early remission received the fewest intravenous cyclophosphamide pulses during induction therapy. The mean oral dose of prednisone (or an equivalent corticosteroid) was 0.6 ± 0.3 mg per kilogram per day from 0 to 3 months and 0.3 ± 0.2 mg per kilogram per day from 4 to 6 months. In the azathioprine group, 11 patients received intravenous methylprednisolone pulses, as compared with 17 in the cyclophosphamide group and 11 in the mycophenolate mofetil group ($P=0.09$).

Remission of nephritis occurred during induction therapy in 49 patients (16 in the azathioprine group, 17 in the cyclophosphamide group, and 16 in the mycophenolate mofetil group). Of the 38 patients with nephrotic proteinuria at base line, 30 had a decrease in the urinary protein:creatinine ratio to less than 3; the decrease was accompanied by an improvement in the base-line serum creatinine level in 18 patients and by a stable serum creatinine level in 12 patients. Of the 21 patients with subnephrotic

proteinuria at base line (a urinary protein:creatinine ratio of less than 3), 19 had a decrease in the urinary protein:creatinine ratio of at least 50 percent; the decrease was accompanied by an improvement in the base-line serum creatinine level in 5 patients and by a stable serum creatinine level in 14 patients. The mean arterial pressure; hematocrit; levels of blood urea nitrogen, serum creatinine, serum albumin, and complement C3 and C4; ANA titer; anti-dsDNA titer; urinary sediment; and urinary protein:creatinine ratio improved significantly with induction therapy in all patients ($P<0.001$).

Table 2 shows the doses of immunosuppressants and corticosteroids received during maintenance therapy. Six patients with persistent nephrotic proteinuria after induction therapy had a remission during maintenance therapy (three in the azathioprine group and three in the mycophenolate mofetil group). The mean dose of intravenous cyclophosphamide and of azathioprine was similar from visit to visit. The median dose of mycophenolate mofetil was 1500 mg per day during the first 12 months and subsequently decreased. The maintenance dose of mycophenolate mofetil was decreased to minimize gastrointestinal side effects and leukopenia in patients who had a sustained remission after a minimum of 12 months of therapy. Overall, during maintenance therapy, the mean dose of prednisone was significantly higher in the cyclophosphamide group (0.21 ± 0.15 mg per day) than in the mycophenolate mofetil group (0.12 ± 0.13 mg per day, $P=0.002$) or the azathioprine group (0.15 ± 0.14 mg per day, $P=0.01$). The amount of corticosteroids used during maintenance therapy was determined on the basis of relapses.

PRIMARY END POINTS

Five patients died during maintenance therapy (four in the cyclophosphamide group and one in the mycophenolate mofetil group). The four patients in the cyclophosphamide group died of sepsis; three of these patients died while still receiving quarterly intravenous pulses of cyclophosphamide. Among these three patients, two died after one and four months and had final nadir white-cell counts of 2600 and 2400 cells per cubic millimeter, respectively. The third patient died after two months and had a final nadir white-cell count of 9200 cells per cubic millimeter. An additional patient died after an uneventful 27-month course of maintenance cyclophosphamide, 10 months after completion of the

Table 1. Characteristics of the Patients at the Beginning of Induction and Maintenance Therapy.*

Characteristic	Before Induction Therapy			Before Maintenance Therapy		
	Azathioprine (N=19)	Cyclophosphamide (N=20)	Mycophenolate Mofetil (N=20)	Azathioprine (N=19)	Cyclophosphamide (N=20)	Mycophenolate Mofetil (N=20)
Age (yr)	33±10	33±12	32±11	—	—	—
No. of women	18	18	19	—	—	—
Race or ethnic group (no.)						
Black	9	9	9	—	—	—
Hispanic	8	11	10	—	—	—
White	2	0	1	—	—	—
Latency (mo)	65±63	35±43	47±54	—	—	—
WHO class (no.)						
III	6	2	4	—	—	—
IV	13	17	16	—	—	—
Vb	—	1	—	—	—	—
Activity index†	7.5±4.8	8.4±5.3	9±6.1	—	—	—
Chronicity index‡	3.2±2.8	1.9±1.5§	3.8±2.8	—	—	—
Nephrotic proteinuria (no.)	14	12	12	3	3	2
Anticardiolipin antibodies (no.)	7	11	10	—	—	—
Hypertension (no.)¶	18	20	19	—	—	—
ACE inhibitor or ARB (no.)	10	9	14	18	14	16
Mean arterial pressure (mm Hg)	103±14	109±18	106±14	92±13	95±16	93±17
Hematocrit (%)	29±5	29±5	31±5	35±3	33±6	34±4
White cells (×10 ⁻³ /mm ³)	7.2±2.4	8.0±4.5	8.6±4.8	5.6±2.6	7.1±4.5	5.6±3.0
Platelets (×10 ⁻³ /mm ³)	236±102	237±120	297±86	267±37	275±87	307±91
Blood urea nitrogen (mg/dl)	43±57	29±25	27±20	16±10	21±12	21±15
Creatinine (mg/dl)	1.7±1.6	1.5±1.2	1.6±1.1	0.96±0.5	0.97±0.5	1.16±0.7
Albumin (mg/dl)	2.7±0.7	2.7±0.7	2.7±0.7	3.5±0.5	3.4±0.5	3.3±0.5
ANA titer						
Median	640	480	640	160	40	40
Range	40–2560	40–2560	160–2560	40–2560	40–640	40–2560
Anti-dsDNA (IU/ml)**	595±817	1157±1592	612±768	102±117	115±128	139±145
C3 (mg/dl)	56±21	61±34	66±29	94±34	96±34	106±29
C4 (mg/dl)	12±4	14±6	14±6	22±8	21±10	24±11
Urinalysis						
White cells (per HPF)	10–25±5–10	10–25±1–5	10–25±5–10	1–5±1–5	5–10±1–5	1–5±0–2
Red cells (per HPF)	10–25±0–2	25–50±1–5	10–25±1–5	1–5±1–5	1–5±1–5	1–5±1–5
Cellular casts (per HPF)	0±0–2	0±0–2	0±0–2	0±0	0±0–2	0±0
Granular casts (per HPF)	1–5±0–2	0–2±0–2	0–2±0–2	0±0–2	0±0–2	0–2±1–5
Protein:creatinine ratio (mg/mg)	5.7±4.6	5.0±3.8	4.7±4.3	1.3±1.4	2.3±3.8	1.5±2.4

* Plus–minus values are means ±SD. Latency is the time between diagnosis of systemic lupus erythematosus and the kidney biopsy. To convert values for blood urea nitrogen to micromoles per liter, multiply by 0.357. To convert values for creatinine to micromoles per liter, multiply by 88.4. WHO denotes World Health Organization, ACE angiotensin-converting–enzyme inhibitor, ARB angiotensin II receptor–blocker, ANA antinuclear antibodies, anti-dsDNA antibodies against double-stranded DNA, and HPF high-power field on light-microscopical examination.

† Scores for the activity index can range from 0 to 24, with higher scores indicating more activity.

‡ Scores for the chronicity index can range from 0 to 12, with higher scores indicating more chronicity.

§ P=0.009 for the comparison with the mycophenolate mofetil group.

¶ Hypertension was defined as a blood pressure of 140/90 mm Hg or more or the use of antihypertensive medications.

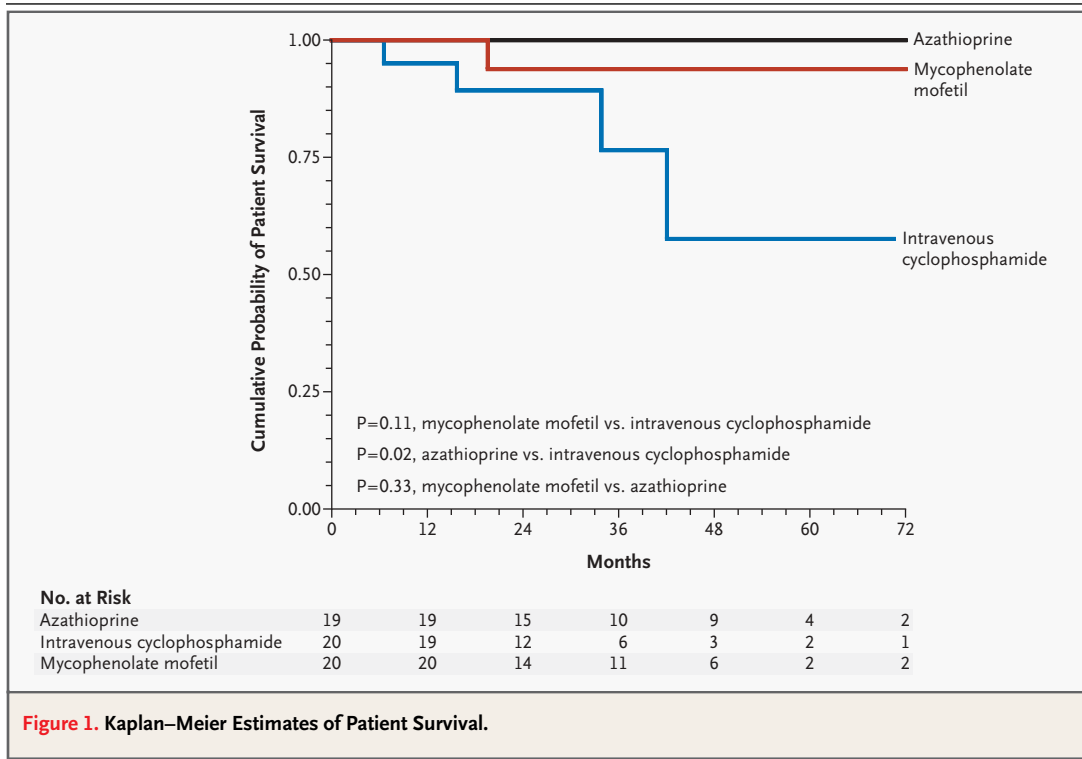
|| P=0.04 for the comparison with the azathioprine group.

** Values were available for 45 patients.

Table 2. Doses of Immunosuppressants and Corticosteroids Received during Maintenance Therapy.*

Time of Visit	Azathioprine Group		Cyclophosphamide Group		Mycophenolate Mofetil Group	
	Azathioprine	Corticosteroids	Cyclophosphamide	Corticosteroids	Mycophenolate Mofetil†	Corticosteroids
	mg/kg/day		mg/m ²	mg/kg/day		mg/day
Mo 0–6	1.2±0.4	0.18±0.11	542±70	0.24±0.19	1500 (1500–2000)	0.18±0.15
Mo 7–12	1.0±0.5	0.11±0.09	565±62	0.18±0.12	1500 (1500–2000)	0.08±0.06‡
Mo 13–18	1.1±0.6	0.11±0.07	562±106	0.20±0.14	1250 (1000–1500)	0.11±0.18
Mo 19–24	0.8±0.6	0.19±0.22	530±119	0.14±0.09	1000 (500–1500)	0.04±0.01‡§
Mo 25–30	1.1±0.5	0.20±0.20	644±4	0.24±0.23	1000 (500–1250)	0.06±0.10
Mo 31–36	1.1±0.6	0.14±0.10	541±36	0.17±0.09	500 (250–500)	0.11–0.04

* Plus-minus values are means ±SD.
 † Values are medians, with 95 percent confidence interval given in parentheses.
 ‡ P=0.02 for the comparison with the cyclophosphamide group.
 § P=0.02 for the comparison with the azathioprine group.



maintenance phase of therapy; this patient had a relapse of autoimmune thrombocytopenia requiring intravenous methylprednisolone that was complicated by sepsis and, subsequently, a cardiac arrest. The patient in the mycophenolate mofetil group died of the acute respiratory distress syndrome secondary to *Pneumocystis carinii* pneumonia. After 10 months of mycophenolate mofetil therapy,

treatment was changed to methotrexate because of a sluggish response of necrotic vasculitis of the soft tissues, and the patient died 5 months later. During maintenance therapy, chronic renal failure developed in five patients: three in the cyclophosphamide group and one each in the azathioprine and mycophenolate mofetil groups. End-stage renal disease developed in one patient after only two

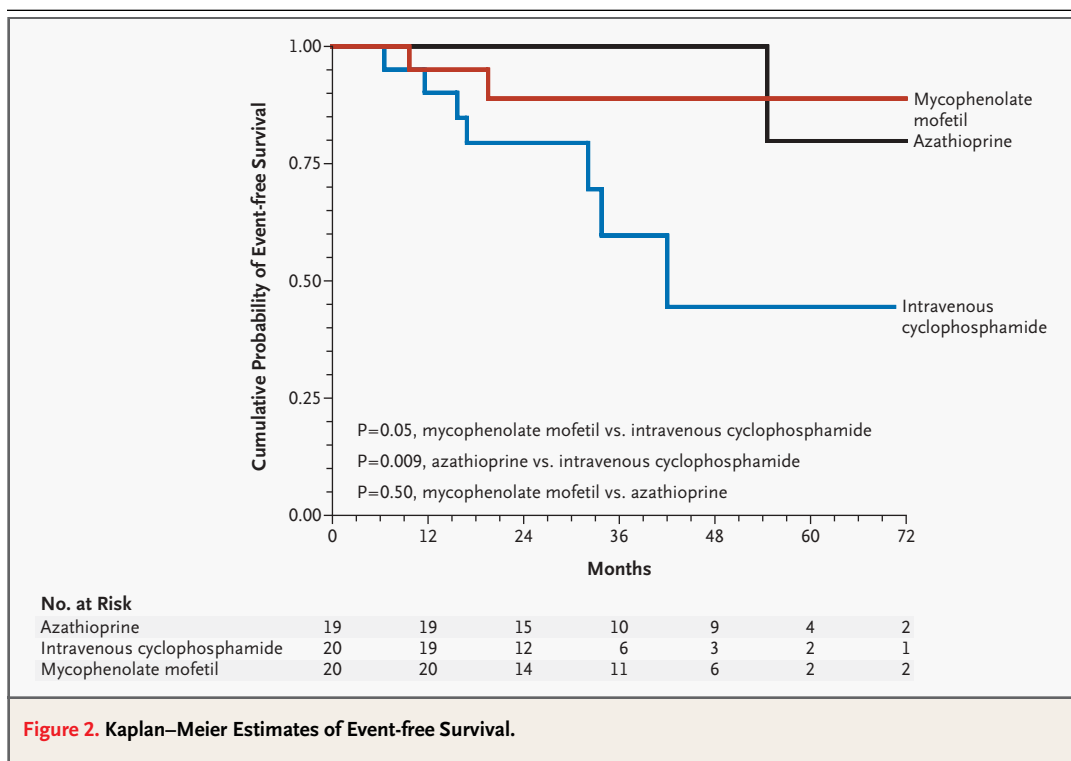


Figure 2. Kaplan–Meier Estimates of Event-free Survival.

weeks of mycophenolate mofetil therapy. The serum creatinine level doubled in two patients in the cyclophosphamide group, and end-stage renal disease subsequently developed. The other two patients (one in the cyclophosphamide group and one in the azathioprine group) had a sustained doubling of the serum creatinine level.

The patient survival rate was higher among patients in the azathioprine group than among those in the cyclophosphamide group ($P=0.02$) (Fig. 1). The cumulative rate of renal survival was similar among the three groups (74 percent in the intravenous cyclophosphamide group, 80 percent in the azathioprine group, and 95 percent in the mycophenolate mofetil group). The event-free survival rate for the composite end point of death or chronic renal failure was higher in the azathioprine and mycophenolate mofetil groups than in the cyclophosphamide group ($P=0.009$ and $P=0.05$, respectively) (Fig. 2).

SECONDARY END POINTS

Renal Relapse

During maintenance therapy, 17 patients had a relapse (8 in the cyclophosphamide group, 6 in the azathioprine group, and 3 in the mycophenolate

mofetil group). All 17 patients had a doubling of the urinary protein:creatinine ratio. The rate of relapse-free survival was higher in the mycophenolate mofetil group than in the cyclophosphamide group ($P=0.02$) (Fig. 3). Six of the 17 relapses were nephritic, with an increase in the serum creatinine level of at least 50 percent for more than one month (3 relapses in the cyclophosphamide group, 2 in the azathioprine group, and 1 in the mycophenolate mofetil group).

ADVERSE EVENTS

The cumulative probability that hospitalization would not be required (excluding elective admissions) was lower in the cyclophosphamide group than in the azathioprine group ($P=0.03$) or the mycophenolate mofetil group ($P=0.007$) during maintenance therapy. The rate was 10 hospital days per patient-year in the cyclophosphamide group, as compared with only 1 hospital day per patient-year in each of the other two groups. There was a significantly higher incidence of sustained amenorrhea in the cyclophosphamide group than in the mycophenolate mofetil or azathioprine group (Table 3). Patients who were randomly assigned to the cyclophosphamide group had a significantly higher total

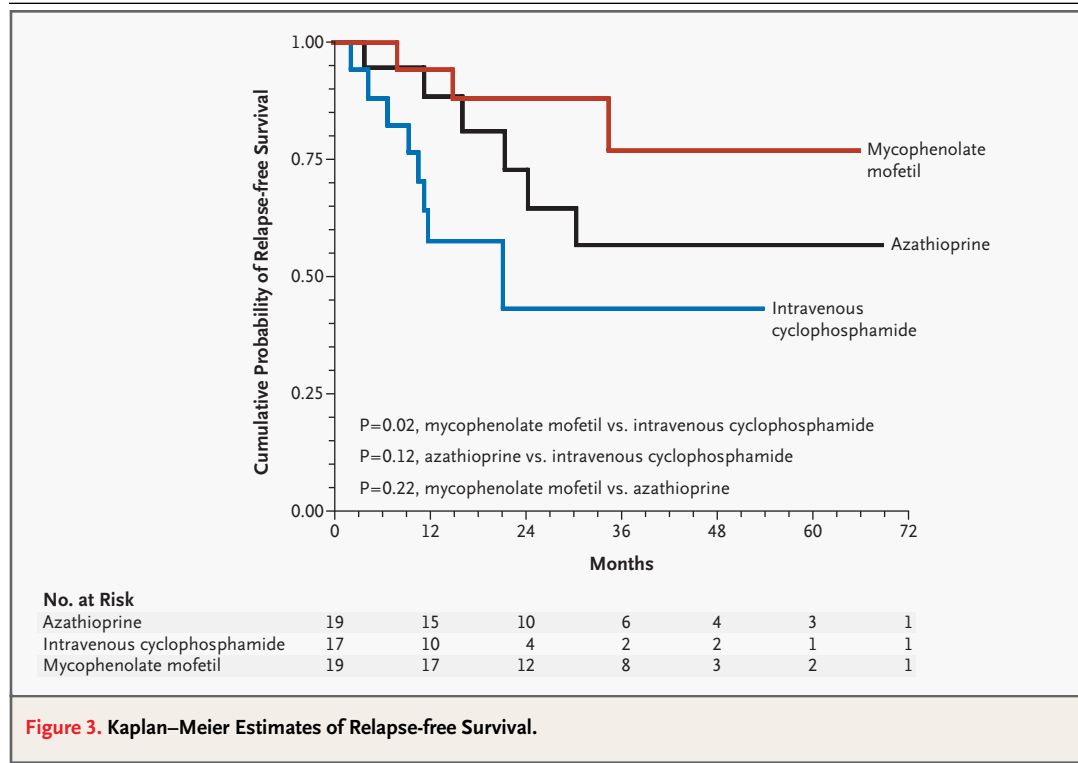


Figure 3. Kaplan–Meier Estimates of Relapse-free Survival.

number of infections and of major infections (pneumonia, sepsis, and meningitis) than did patients in the other two groups. The frequency of nausea and vomiting was significantly higher in the cyclophosphamide group than in the other two groups. The incidence of leukopenia was similar among the three groups (Table 3). There were no episodes of hemorrhagic cystitis or cases of cancer during the study.

DISCUSSION

In the past decade, we and others^{9,19} have assessed the efficacy and safety of newer immunosuppressive regimens for the treatment of proliferative lupus nephritis. We designed the immunosuppressive protocols used in the current study with the goal of lessening the considerable toxic effects of long-term therapy with cyclophosphamide, since this drug was found to be the most effective immunosuppressive agent in studies by the National Institutes of Health (NIH).²⁻⁶

Chan et al.⁹ reported that mycophenolate mofetil was as effective in inducing remission at 12 months as was 6 months of oral cyclophosphamide followed by azathioprine in a population of

Asian patients with diffuse proliferative glomerulonephritis. Eighty-one percent of the patients in the mycophenolate mofetil group had a complete remission, as did 76 percent of those in the sequential-therapy group. Only two patients died in the sequential-therapy group. Amenorrhea developed only among patients in the sequential-therapy group (incidence, 23 percent). Infections developed in 26 percent of the patients overall (19 percent of those in the mycophenolate mofetil group and 33 percent of those in the sequential-therapy group).⁹

In a clinical trial involving a predominantly white population, Houssiau et al.¹⁹ found that two sequential immunosuppressive regimens — low-dose intravenous cyclophosphamide (six pulses of 0.5 g every two weeks) followed by maintenance therapy with azathioprine and high-dose intravenous cyclophosphamide (six monthly pulses of 0.5 g per square meter and two quarterly pulses adjusted according to the nadir leukocyte count) followed by maintenance therapy with azathioprine — were similarly efficacious, with low rates of treatment failure and chronic renal failure. Only two patients died in the group given low-dose intravenous cyclophosphamide. Sustained amenorrhea occurred in 4 percent of the patients in each group. The rates of

Table 3. Rates of Amenorrhea, Infections, and Other Adverse Events during Maintenance Therapy.*

Adverse Event	Azathioprine Group	Mycophenolate Mofetil Group	Cyclophosphamide Group	P Value for Comparison with Azathioprine	P Value for Comparison with Mycophenolate Mofetil
	<i>percent</i>				
Amenorrhea†	8	6	32	0.03	0.03
Infection					
Total	29	32	77	0.002	0.005
Major	2	2	25	0.01	0.02
Pneumonia	2	2	15	0.05	0.06
Sepsis with bacteremia	0	0	8	—	—
Meningitis	0	0	3	—	—
Minor	28	30	52	0.06	0.11
Upper respiratory tract	22	14	32	0.34	0.08
Urinary tract	2	10	3	0.83	0.20
Herpes zoster	4	6	17	0.05	0.13
Leukopenia‡	6	2	10	0.43	0.15
Nausea§	7	14	65	<0.001	<0.001
Vomiting§	4	10	55	<0.001	<0.001
Diarrhea	9	12	12	0.97	0.63

* Unless otherwise noted, rates of adverse events were calculated as $100 \times (\text{the number of events} \div \text{the number of patient-years of follow-up})$.

† Rates of sustained amenorrhea were calculated as $100 \times (\text{the number of patients} \div \text{the number of patient-years of follow-up})$, with censoring of data in June 2002.

‡ Leukopenia was defined as a white-cell count of less than 2000 per cubic millimeter.

§ Nausea and vomiting included events that occurred after the infusion of cyclophosphamide.

severe infection were 15 percent in the group given low-dose cyclophosphamide and 25 percent in the group given high-dose cyclophosphamide.¹⁹

In our study, short-term treatment with intravenous cyclophosphamide followed by maintenance therapy with either mycophenolate mofetil or azathioprine resulted in a lower rate of death or chronic renal failure than did long-term therapy with intravenous cyclophosphamide. In contrast to the NIH trials,²⁻⁵ in our study, patients receiving long-term intravenous cyclophosphamide had a lower cumulative probability of remaining free of chronic renal failure (74 percent, as compared with more than 85 percent) and relapse (43 percent, as compared with more than 85 percent). It is important to note that our study included predominantly high-risk Hispanic²⁰ and black²¹ patients, as opposed to the predominantly white population in the NIH trials.²⁻⁵

We also demonstrated that short-term administration of intravenous cyclophosphamide followed by mycophenolate mofetil or azathioprine was safer than long-term therapy with intravenous cyclophosphamide. The hospitalization rates were significant-

ly lower in both sequential-therapy groups than in the group given long-term intravenous cyclophosphamide. Rates of sustained amenorrhea in the sequential-therapy groups were similar to those in the two sequential-therapy groups in the study by Houssiau et al., in which the use of intravenous cyclophosphamide was limited to eight pulses.¹⁹ The group that received long-term intravenous cyclophosphamide in our study had an incidence of sustained amenorrhea of 32 percent, which is within the range of 29 to 57 percent in the groups that received long-term intravenous cyclophosphamide in the NIH studies.²⁻⁶ The incidence of severe infections was significantly lower in the two sequential-therapy groups than in the group given long-term intravenous cyclophosphamide.

In summary, short-term therapy with intravenous cyclophosphamide followed by maintenance therapy with mycophenolate mofetil or azathioprine was more efficacious and safer than long-term therapy with intravenous cyclophosphamide for the treatment of proliferative lupus nephritis. Maintenance therapy with mycophenolate mofetil was as-

sociated with a significantly lower relapse rate than was long-term therapy with intravenous cyclophosphamide. Our study was not powered to detect small differences between the two sequential-therapy groups. In addition, our results cannot be generalized to children with lupus nephritis or patients

with mild forms of lupus nephritis, since such patients were excluded from our trial.

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APPENDIX

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REFERENCES

1. Appel GB, Cohen DJ, Pirani CL, Meltzer JI, Estes D. Long-term follow-up of patients with lupus nephritis: a study based on the classification of the World Health Organization. *Am J Med* 1987;83:877-85.
2. Austin HA III, Klippel JH, Balow JE, et al. Therapy of lupus nephritis: controlled trial of prednisone and cytotoxic drugs. *N Engl J Med* 1986;314:614-9.
3. Steinberg AD, Steinberg SC. Long-term preservation of renal function in patients with lupus nephritis receiving treatment that includes cyclophosphamide versus those treated with prednisone only. *Arthritis Rheum* 1991;34:945-50.
4. Boumpas DT, Austin HA III, Vaughan EM, et al. Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. *Lancet* 1992;340:741-5.
5. Gourley MF, Austin HA III, Scott D, et al. Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis: a randomized, controlled trial. *Ann Intern Med* 1996;125:549-57.
6. Boumpas DT, Austin HA III, Vaughan EM, Yarboro CH, Klippel JH, Balow JE. Risk for sustained amenorrhea in patients with systemic lupus erythematosus receiving intermittent pulse cyclophosphamide therapy. *Ann Intern Med* 1993;119:366-9.
7. Lewis EJ, Hunsicker LG, Lan S-P, Rohde RD, Lachin JM. A controlled trial of plasmapheresis therapy in severe lupus nephritis. *N Engl J Med* 1992;326:1373-9.
8. Chan T-M, Li F-K, Wong RWS, Wong K-L, Chan K-W, Cheng IKP. Sequential therapy for diffuse proliferative and membranous lupus nephritis: cyclophosphamide and prednisolone followed by azathioprine and prednisolone. *Nephron* 1995;71:321-7.
9. Chan TM, Li FK, Tang CSO, et al. Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. *N Engl J Med* 2000;343:1156-62.
10. Eugui EM, Mirkovich A, Allison AC. Lymphocyte-selective antiproliferative and immunosuppressive effects of mycophenolic acid in mice. *Scand J Immunol* 1991;33:175-83. [Erratum, *Scand J Immunol* 1998;48:45.]
11. Corna D, Morigi M, Facchinetti D, Bertani T, Zoja C, Remuzzi G. Mycophenolate mofetil limits renal damage and prolongs life in murine lupus autoimmune disease. *Kidney Int* 1997;51:1583-9.
12. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
13. Lupus nephritis, lupus-like syndrome, antiphospholipid antibody syndrome. In: Churg J, Bernstein J, Glasscock RJ. *Renal disease: classification and atlas of glomerular disease*. 2nd ed. New York: Igaku-Shoin, 1995:151-79.
14. Pollak VE, Pirani CL, Schwartz FD. The natural history of the renal manifestations of systemic lupus erythematosus. *J Lab Clin Med* 1964;63:537-50.
15. Austin HA III, Muenz LR, Joyce KM, Antonovych TT, Balow JE. Diffuse proliferative lupus nephritis: identification of specific pathologic features affecting renal outcome. *Kidney Int* 1984;25:689-95.
16. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457-81.
17. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep* 1966;50:163-70.
18. Inference in parametric models and related topics: comparison of two exponential samples. In: Kalbfleisch JD, Prentice RL. *The statistical analysis of failure time data*. New York: John Wiley, 1980:39-68.
19. Houssiau FA, Vasconcelos C, D'Cruz D, et al. Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-dose versus high-dose intravenous cyclophosphamide. *Arthritis Rheum* 2002;46:2121-31.
20. Alarcon GS, McGwin G Jr, Petri M, Revell JD, Ramsey-Goldman R, Kimberly RP. Baseline characteristics of a multiethnic lupus cohort: PROFILE. *Lupus* 2002;11:95-101. [Erratum, *Lupus* 2002;11:402.]
21. Dooley MA, Hogan S, Jennette C, Falk R. Cyclophosphamide therapy for lupus nephritis: poor renal survival in black Americans. *Kidney Int* 1997;51:1188-95.

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