Concise Report

Long-term effects of combination treatment with fludarabine and low-dose pulse cyclophosphamide in patients with lupus nephritis

G. G. Illei¹, C. H. Yarboro¹, T. Kuroiva¹, R. Schlimgen¹, H. A. Austin², J. F. Tisdale², P. Chitkara¹, T. Fleisher³, J. H. Klippel¹, J. E. Balow⁴ and D. T. Boumpas¹,⁴

Objectives. To determine the safety and efficacy of a short course of fludarabine combined with cyclophosphamide in lupus nephritis.

Methods. A phase I/II open label pilot study. Thirteen patients with active proliferative lupus nephritis received monthly oral boluses of low-dose cyclophosphamide (0.5 gm/m² on day 1) and subcutaneous fludarabine (30 mg/m² on days 1–3) for 3–6 cycles. Concomitant prednisone was aggressively tapered from 0.5 mg/kg/day to a low-dose, alternate-day schedule. Patients were followed for at least 24 months after therapy. The primary outcome was the number of patients achieving renal remission defined as stable creatinine, proteinuria <1 gm/day and and inactive urine sediment for at least 6 months.

Results. The study was terminated early because of bone marrow toxicity. Eleven patients who received at least three cycles were evaluated for efficacy. Ten patients improved markedly with seven patients achieving complete remission and three patients achieving partial remission. There were three serious haematological adverse events during the treatment with one death due to transfusion-associated graft vs host disease. Profound and prolonged CD4 (mean CD4: 98/μl at 7 months and 251/μl at 12 months) and CD20 lymphocytopenia was noted in most patients. Three patients developed Herpes zoster infections.

Conclusions. A short course of low-dose fludarabine and cyclophosphamide can induce long-lasting remissions in patients with proliferative lupus nephritis, but severe myelosuppression limits its widespread use.

KEY WORDS: systemic lupus erythematosus, chemotherapy, myelosuppression, remission, safety.

Introduction

Several clinical trials have demonstrated the efficacy of cyclophosphamide in preserving renal function. However, even with a long course (30 months or longer) of therapy, up to 40% of patients fail to achieve renal remission and to 20% of patients develop end-stage renal disease (ESRD) [1–4].

Cyclophosphamide can be associated with adverse effects which have to be weighed against morbidity occurring as a result of uncontrolled disease and the need for extended therapy. The risk of premature ovarian failure, a common long-term side effect, is dependent on the age of the patient at the initiation of therapy and the cumulative dose of cyclophosphamide [5–7]. Combining cyclophosphamide with drugs that have additive effects but a different side-effect profile may allow the reduction of the cumulative dose of cyclophosphamide and decrease the risk of amenorrhea without loss of efficacy.

Fludarabine is a halogenated adenosine analog, used in the treatment of haematological malignancies. It induces profound and prolonged immunosuppression by depleting both T- and B-lymphocytes [8] and inhibiting cytokine-induced activation of STAT1 in resting as well as activated lymphocytes [9], which may contribute to the immunosuppressive effect of fludarabine.

In a Phase 1 study, continuous 7-day infusion of 2-CdA, another adenosine analogue with a similar mechanism of action, led to complete response in 3/7 patients and all 7 had maintained stable renal function during the 1-yr follow-up [10] and 6-month course of fludarabine led to improvement in proteinuria and filtration rate in a pilot study in membranous nephritis [11]. In vitro studies [12] demonstrating a synergistic action of fludarabine and cyclophosphamide were supported by clinical studies in chronic lymphocytic leukaemia (CLL) [13]. Based on these experiences, we conducted a pilot study in lupus nephritis to evaluate the safety and to collect preliminary data about the efficacy of a short course of low-dose cyclophosphamide combined with fludarabine.

Materials and methods

Study design

This was single centre, phase I/II, open label pilot study. We intended to treat 15 patients with proliferative lupus nephritis. The Institutional Review Board of the National Institute of Allergy and Infectious Diseases, NIH approved the study. Written informed consent was obtained from all patients.

Patient selection

Thirteen patients older than 18 yrs and with a diagnosis of SLE according to the 1982 criteria of the American College of Rheumatology were enrolled. Inclusion criteria included a renal biopsy showing proliferative lupus nephritis within 1 year prior to first dose of study drug and (i) >10 RBC/hpf and cellular (RBC, WBC or mixed) casts, or (ii) >10 RBC/hpf and proteinuria >2 g/day, or (iii) proteinuria >3.5 g/day.

Exclusion criteria included severe renal disease (rapidly progressive glomerulonephritis or fibrinoid necrosis and/or cellular crescents affecting >25% of glomeruli); severe impairment of renal function (serum creatinine >2.5 mg/dl or GFR<50 ml/min measured by inulin clearance); treatment with azathioprine, cyclosporine or methotrexate within 4 weeks; prior exposure to substantial dose of cyclophosphamide (>3 pulses (maximum
Fludarabine and cyclophosphamide in lupus nephritis

1 g/m²/pulse) within the last 6 months or since last renal biopsy showing active disease or ≥4 pulses, unless there is a biopsy showing active disease after a period of ≥6 months since last treatment; pulse therapy with glucocorticoids or any experimental therapy during the 4 weeks before study entry or need at study entry of oral corticosteroids in dosages >0.5 mg/kg/day of prednisone to control extrarenal disease; active or chronic infection; pregnancy, breast-feeding or inadequate birth control; history of cerebrovascular accident, seizures within the last 5 years or chronic neurological disease; history of malignancy other than squamous cell and/or basal carcinoma of the skin and haematological disease (haemoglobin <8 mg/dl, platelets <50,000/µl or WBC <1500/µl).

Following a serious adverse event in patient #4, the exclusion criteria about past cyclophosphamide use and abnormal haematological values were changed. Subjects were excluded if they received >3 pulses of cyclophosphamide (maximum 1 g/m²/pulse) within the last 12 months or since last renal biopsy showing active disease or >6 pulses ever. The exclusion criteria for abnormal haematological values were changed to: haemoglobin <8 mg/dl, platelets <100,000/µl or WBC <2500/µl.

Concomitant therapy
All patients received oral prednisone 0.5 mg/kg/day for 4 weeks. Prednisone dose was then tapered by 5 mg every other day each week to 0.25 mg/kg every other day. After month 6, prednisone was tapered at the treating physician’s discretion to low-dose alternate-day regimen.

Prophylactic therapy
All patients were vaccinated with pneumococcal, haemophilus influenzae B (HiB), influenza (during influenza season) and tetanus toxoid vaccines. Patients received Pneumocystis carinii and herpes prophylaxis based on their absolute lymphocyte counts.

Study treatment
Patients received monthly cycles of cyclophosphamide 500 mg/m² orally on day 1 followed by fludarabine 30 mg/m² subcutaneously (SQ) on days 1–3 for 6 monthly cycles. The dose of fludarabine and/or cyclophosphamide was reduced according to predetermined criteria for severe lymphopenia, leukopenia or thrombocytopenia. The protocol was modified to decrease the number of cycles to 3 after patient #4 developed severe aplastic crises after the third cycle.

Outcomes and data analysis
The primary outcome was the determination of safety (rates of serious adverse events and infections) and tolerability. Secondary outcomes included assessment of the rate of amenorrhea and renal efficacy measures: renal remission was defined as proteinuria <1 gm/day, inactive urine sediment (<10 red blood cells/hpf and no cellular casts in the sediment of a 50 ml urine sample) and stable serum creatinine. Partial response was defined as ≥50% reduction in 24h proteinuria, if baseline >2 g/day, with persistently active urine sediment; or inactive urine sediment (if active at baseline) with proteinuria <2 g/day if nephrotic at baseline (or ≤50% of baseline if non-nephrotic). All patients were included in the toxicity analysis. Patients must have completed 3-cycles of therapy to be included in the analysis for efficacy.

The primary and secondary outcomes were determined at 12 months. All patients were regularly followed for at least 18 months after that to establish long-term effects of therapy; over 5 years of follow-up data are available for all but one.

Statistical analysis
All P-values are two-sided. Averages are expressed as the mean and standard deviation. Changes in the absolute values and percentage change from baseline at different time-points were compared by repeated measures analysis of variance (ANOVA). Adjustments for multiple comparisons were made using Scheffe’s method. All statistical analyses were done with the Statview V.5 statistical software package (SAS Institute, Cary, NC).

Results
Patient characteristics
Thirteen patients entered the protocol. A significant number of patients exhibited features that have been associated with poor renal outcomes such as male gender (3 patients), African American ethnicity (5 patients) and diffuse proliferative (7 patients) or mixed membranous and proliferative glomerulonephritis (4 patients). The majority of patients (10/13) had nephrotic range proteinuria; 5 had anti-dsDNA antibodies and 7 of the 13 had low C3 concentrations. Only two patients received more than six cycles of cyclophosphamide pulses before entering the study.

Adverse events
Clinically significant adverse events are summarized in Table 1. There were three serious haematological adverse events during the treatment period. Patient #4 developed aplastic crisis with neutropenic fever 1 week after receiving the third cycle. She eventually died of a transfusion-associated graft versus host-like disease (TA-GVHD), as reported earlier [14]. Following this episode of aplastic crisis, the protocol was revised and the number of treatment cycles was reduced from six to three. Two other patients experienced Grade 4 neutropenia; neither of these was associated with any clinical complications and both patients recovered spontaneously (Table 1). Bone marrow biopsy was normal in both patients. However, based on the unexpected rate and severity of bone marrow suppression, the study was terminated early. Both of these patients along with a third patient had intermittent leukopenia during their long-term follow-up, with no clinical sequelae.

Two patients had immune mediated thrombocytopenia and three patients had Herpes zoster infections; two of them had history of herpes infections and the third patient developed it 1 week after the first study treatment. No other serious infections were observed.

Renal outcome
A total of 11 patients who received at least three cycles were evaluated for efficacy (Table 1 and Fig. 1).

Proteinuria. Proteinuria improved rapidly and substantially in all patients (Fig. 1A). By 6 months, mean proteinuria decreased to 2.1 gram/day from 5.6 gram/day at baseline with all but two patients having at least 50% improvement. At 12 months, mean proteinuria was 0.9 gm/day, all patients had more than 70% improvement, with 7/11 patients having <1 gram/day protein excretion.

Remissions. By 6 months nine patients achieved at least partial response (Fig. 1B) despite aggressive tapering of steroids (mean daily dose ± SD: 7.9 ± 1.9 mg vs 27.5 ± 9.6 mg at baseline). At 12 months, four patients were in remission; all other patients met criteria for partial response. Three of those with partial remission were treated with additional immunosuppressive therapy for ongoing renal disease. Two of these patients had biopsies after the protocol; both showed mild focal
<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Cycles(#)</th>
<th>Treatment phase</th>
<th>Renal response</th>
<th>Complications</th>
<th>SLE DAI</th>
<th>Renal response</th>
<th>Complications</th>
<th>SLE DAI</th>
<th>F/U time</th>
<th>Renal response</th>
<th>Complications</th>
<th>SLE DAI at last F/U</th>
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</thead>
<tbody>
<tr>
<td>01</td>
<td>06</td>
<td>PR after 1st cycle</td>
<td>None</td>
<td>4 CR at 15 months</td>
<td>Leucopenia (Grade 2) 2 months after last treatment, normal BM biopsy</td>
<td>None</td>
<td>CR at 21 months</td>
<td>None</td>
<td>1</td>
<td>CR</td>
<td>Leucopenia (Grade 2) 2 months after last treatment, normal BM biopsy</td>
<td>None</td>
</tr>
<tr>
<td>02</td>
<td>06</td>
<td>PR after 1st cycle</td>
<td>None</td>
<td>6 CR at 21 months</td>
<td>None</td>
<td>CR at 21 months</td>
<td>None</td>
<td>0</td>
<td>CR</td>
<td>Intermittent leucopenia</td>
<td>None</td>
<td>CR</td>
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<td>05</td>
<td>PR after 3 cycles, CR at 6 months</td>
<td>None</td>
<td>1 CR</td>
<td>None</td>
<td>CR</td>
<td>None</td>
<td>0</td>
<td>CR</td>
<td>None</td>
<td>None</td>
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<tr>
<td>04</td>
<td>03</td>
<td>Aplastic crisis after 3rd cycle with death from TA-GVHD (recol non-irradiated platelet and RBC transfusions)</td>
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<td>2 CR</td>
<td>Anterior uveitis at 7 months, treated with topical steroids.</td>
<td>None</td>
<td>CR</td>
<td>0</td>
<td>CR</td>
<td>Thyroiditis</td>
<td>None</td>
<td>CR</td>
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<td>04</td>
<td>PR after 1st cycle, CR at 6 months</td>
<td>Herpes zoster reactivation after 1st cycle, erythematous rash at 5 months, improved with prednisone.</td>
<td>0 CR</td>
<td>None</td>
<td>CR</td>
<td>None</td>
<td>2</td>
<td>CR</td>
<td>None</td>
<td>None</td>
<td>0</td>
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<tr>
<td>06</td>
<td>03</td>
<td>CR after 1st cycle</td>
<td>None</td>
<td>4 CR</td>
<td>CyA 12-27 months, no response</td>
<td>Mucocutaneous rash</td>
<td>4</td>
<td>CR</td>
<td>12</td>
<td>CR</td>
<td>Mucocutaneous rash</td>
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<td>07</td>
<td>03</td>
<td>PR at 3 months</td>
<td>None</td>
<td>5 PR, contd on prednisone</td>
<td>None</td>
<td>CR at 21 months</td>
<td>None</td>
<td>0</td>
<td>CR</td>
<td>None</td>
<td>None</td>
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<tr>
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<td>PR after 1st cycle</td>
<td>Pancreatic cyst as a complication of pre-existing pancreatitis after 2nd cycle</td>
<td>2 CR</td>
<td>PR, contd on prednisone</td>
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<td>CR</td>
<td>0</td>
<td>CR</td>
<td>None</td>
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<td>03</td>
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<td>PR, started on MMF at 2 months, went to CR at 12 months</td>
<td>None</td>
<td>CR</td>
<td>2</td>
<td>CR</td>
<td>None</td>
<td>None</td>
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<tr>
<td>10</td>
<td>03</td>
<td>PR after 1st cycle</td>
<td>Herpes zoster 1 week after 1st dose</td>
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<td>Leucopenia (Grade 4) 2 months after 3rd cycle, herpes zoster after 2nd cycle</td>
<td>None</td>
<td>CR at 12 months</td>
<td>0</td>
<td>CR</td>
<td>None</td>
<td>None</td>
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<tr>
<td>11</td>
<td>03</td>
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<td>Relapsed into PR at 12 months, CR at 18 months</td>
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<td>CR</td>
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<td>None</td>
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<tr>
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<td>Post 2 doses, switched to monthly CYC with CR</td>
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<td>CR</td>
<td>Intermittent leucopenia</td>
<td>0</td>
<td>CR</td>
<td>0</td>
<td>CR</td>
<td>Mucocutaneous disease, intermittent leucopenia</td>
<td>None</td>
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<tr>
<td>13</td>
<td>02</td>
<td>Post 2 doses, switched to PO CYC with PR.</td>
<td>Leucopenia (Grade 3) 2 months after 2nd cycle</td>
<td>4</td>
<td>Monthly IV CYC started at 16 months, with CR</td>
<td>Herpes zoster at 20 months</td>
<td>None</td>
<td>2</td>
<td>CR</td>
<td>None</td>
<td>None</td>
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</tr>
</tbody>
</table>

CR, Complete Remission; PR, partial response; CYC, cyclophosphamide; MMF, mycophenolate mofetil; CyA, cyclosporine; AZA, azathioprine; AVM, arterio-venous Malformation; OSA, obstructive sleep apnea; CPAP, continuous positive airway pressure; F/U, follow up; IVIg, intravenous immunoglobulin.
Lymphodepletion was achieved promptly in all patients. CD4

Immunological effects

Three patients had a total of five uncomplicated pregnancies with term deliveries. Three patients had a total of five uncomplicated pregnancies with term deliveries. Amenorrhoea

None of the patients developed amenorrhoea during the study. Three patients had a total of five uncomplicated pregnancies with term deliveries.

Immunological effects

Lymphodepletion was achieved promptly in all patients. CD4+ T cells recovered more slowly than CD8+ T cells and B cells. The delayed recovery was most pronounced in activated T cells expressing the IL-2 receptor (CD4+ CD25+); this, however, was not associated with an increase in infectious complications (Fig. 1).

Discussion

In this pilot study, a limited course of subcutaneous fludarabine in combination with low-dose oral pulse cyclophosphamide was associated with an unexpectedly high rate of haematological side effects. Despite some encouraging preliminary efficacy data the risk-benefit assessment of this treatment in its current format is unfavourable.

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The authors have declared no conflicts of interest.

References