sion of MDM2 and p53 mRNA and could not detect different MDM2 mRNA levels based on the MDM2-309 genotype, suggesting that MDM2 levels are controlled independently in CLL.

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A possible role for low-dose cyclosporine in refractory immune thrombocytopenic purpura

Management of patients with severe, refractory, chronic immune thrombocytopenic purpura (ITP) is still difficult. However, although there is no consensus on the best treatment strategy, these patients show a persistent, marked thrombocytopenia, and need therapy, owing to the presence of or to an increased risk of bleeding. Various treatments have been attempted: immunosuppressive chemotherapy, high-dose dexamethasone, danazol, combination chemotherapy, which have shown transient response in a variable percentage of cases, but none with any evidence of safe and durable efficacy.[1] Recently, a systematic review of more than 300 patients treated with a monoclonal anti-CD20 antibody, rituximab, many of whom with a severe form of ITP, has shown an overall platelet response of 62.5%, a median response duration of 10.5 months, but also significant toxicities. Therefore, the optimal timing and dose of the drug remain undefined.[2] More recently, good results have been reported with a multiagent induction and maintenance therapy, even if the duration of response was not defined.[3] Furthermore, an increase in platelet counts has been obtained using a thrombopoietin-receptor agonist, eltrombopag, though the durability of the response and the long-term safety of this compound are unknown.[4] Finally, a promising further approach seems to be the active and safe use of low dose rituximab.[5] As reported in published data, there are considerable side-effects associated with the current treatment and the responses of various therapies have not yet been consolidated. Other therapeutic strategies should, therefore, also be considered. There are few data in the literature describing the effects of cyclosporine (CyA) therapy in this setting. A study has been reported in adults in whom high toxicity offsets benefits,[6] and this finding has also been confirmed in children.[7] However, in both studies high doses of CyA (5-10 mg/kg/d) have been used.

We have already reported on long-term salvage therapy with CyA in 12 severe, refractory, chronic ITP[8] with 83.3% of response (10/12), lasting for a median follow-up of 36.8 months. The patients were 9 women and 3 men (median age 66.6 years, range 42-85 years). All patients had previously received 2-3 drug therapies and 8 patients had also undergone splenectomy. Only patients with platelet counts less than 30×10^9/L entered the study. All patients had major or minor bleeding episodes, often transient, but recurrent. Results of a long-term follow-up of the responsive patients are shown in Table 1. The updated median follow-up of approximately 5.5 years (69 months, range 4-13 years), shows that 9 patients had maintained response for the duration of the observation period (Figure 1). A further patient, not considered for the follow-up (n.8) died in complete remission of myocardial infarction during a 3-month course of CyA treatment. Five patients (ns. 1,2,7,9,and 10) had a complete response (platelet counts in normal range), one patient (n. 5) had a partial response (platelet counts between 80 and $150\text{x}10^9$/L), and 2 patients (ns. 3 and 12) had a complete response which had been maintained with continued drug administration. One patient (n. 4) had a drug-dependent complete response for approximately 4.5 years and a partial response for a further 1.5 year, after CyA had been tentatively discontinued and

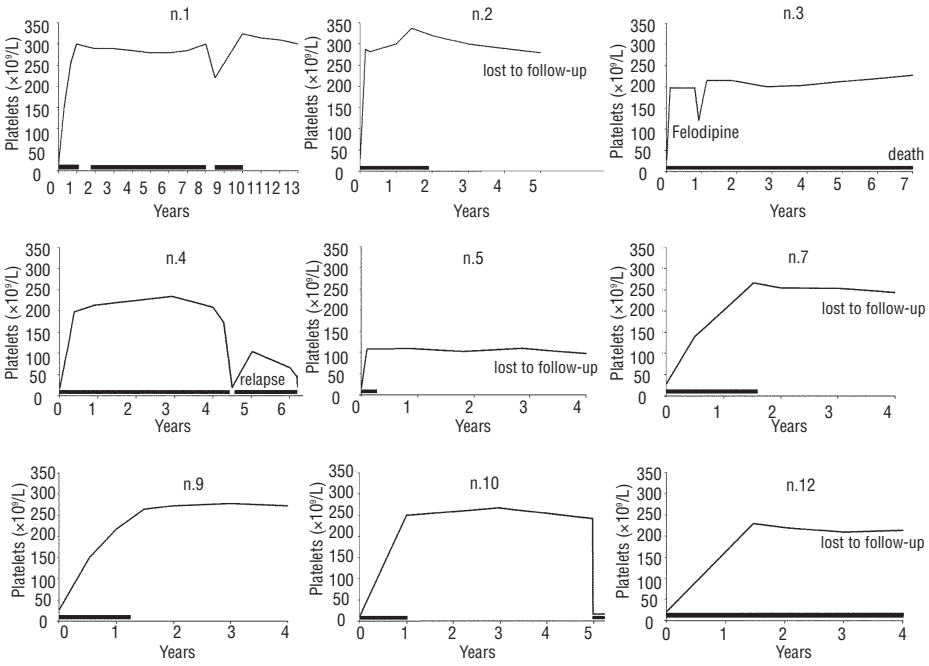

Figure 1. Updated long-term follow-up of platelet counts in patients treated with CyA. Follow-up from April 1994 to May 2007. Each small number is referred to one responsive patient identified by number in the previous report.[8] The bars indicate the time of CyA administration. Patients ns. 3 and 12 had maintained response with continued administration of CyA. In patient n. 4 a decrease of platelet counts was noted when CyA had been tentatively discontinued. In patient n. 3, a decrease in platelet counts was found concomitantly with administration of felodipine.

Table 1. Responsive patients' characteristics.

Patient n.	*Previous therapies*	*Platelets (x10⁹/L) before CyA*	*Bleeding before CyA*	*CyA complications*
1	P,G,A,Sple	15	++	Fatigue, hypertension
2	P,G,ALG,Sple	29	++	Gingival hyperplasia
3	P,G,ALG,Sple	18	+	Hypertrichosis, increased creatinine
4	P,G,A,Sple	20	++	Hypertension, Fatigue
5	P,G,C,Sple	10	++	Gingival hyperplasia, tremor
7	P,G,C	28	++	Hypertension, candidiasis
9	P,G,C	27	++	Gingival hyperplasia
10	P,G,Sple	12	++	Myalgia
12	P,G,C	25	+	Paresthesias

P: indicates prednisone; G: γ-globulins; A: azathioprine; ALG: antilymphocyte globulin; C: cyclophosphamide; Sple: splenectomy. ++ indicates major bleeding (diffuse ecchymosis and/or intrabuccal hemorrhagic vesicles, prolonged epistaxis, menorrhagia, gastrointestinal, and/or genitourinary bleeding); + indicates minor bleeding (mild purpura, mild epistaxis, gingival bleeding, easy bruising).

then restored. Two patients (ns. 10 and 4) had a relapse at 5 and 6.5 years, respectively, and 1 patient (n. 3) died at seven years. The drug dose was 2.5-3 mg/kg/d, after a starting dose of 5 mg/kg/d for six days, to maintain a therapeutic serum level between 200 and 400 ng/mL. Patient monitoring included monthly blood cell counts and examination of CyA serum level, renal and hepatic functions every week for one month, then every two months and every six months, after a stable remission had been achieved. Platelet counts usually began to increase after 3-4 weeks of treatment. A further reduction of daily dose of CyA could be obtained by the co-administration of grapefruit juice. The side-effects were minor, transient and reversible, even after long-term, continued treatment. Intolerance manifestations usually resolved spontaneously or with a dose reduction of CyA or its withdrawal for a few days. Only an infectious episode (candidiasis, pt. n. 7) was recorded, which was rapidly resolved by standard antifungal therapy and discontinuation of CyA for few days. Only one patient (n. 3) showed a creatinine increase exceeding 150% of the baseline value, resolved by a CyA dose reduction of 25% for ten days. Similar results were recently obtained in adults, in a smaller series with shorter follow-up, by others[9] and also in children.[10] Furthermore, CyA has recently been recommended as a second-line therapy in Evans' syndrome.[11]

The persistently high rate of responses, and the low and transient toxicities, recorded after such a long-term follow-up, suggest that the oral administration of low-dose CyA, could be considered in severe, refractory ITP as a salvage therapy and/or as a maintenance treatment after a response has been obtained by rituximab or more aggressive approaches.

We have been using this approach in our current clinical practice and believe that the debated issue of CyA-related toxicity largely relies on the use of higher doses of the drug and can be otherwise easily managed by the use of standard or even lower doses of the drug and by a routine assessment of patients' clinical and laboratory parameters. Therefore, we encourage further studies on larger series of patients to assess the general relevance of our findings. We also suggest that patients with refractory ITP, screened positive for *Helicobacter pylori* (Hp) infec-

tion should undergo bacterium eradication before or after CyA as reported,[12] at least in areas with a high prevalence of Hp infection.

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IgM anti-protein S antibodies as a risk factor for venous thrombosis

Lupus anticoagulant (LA) and antiphospholipid antibodies (APA) are immunoglobulins directed at phospholipid-protein complexes that have been associated frequently with thrombophilia. Protein S (PS) is a protein with high affinity for phospholipids and can be a target for APA. It is a cofactor in the Protein C (PC) anticoagulant pathway and facilitates the inactivation of coagulation Factors Va and VIIIa. We performed a case-control study to assess the prevalence of anti-protein S antibodies (anti-PS) in a Spanish population and to determine if they are a risk factor for venous thrombosis (VT).

Patients were included when they had been referred to our hospital from November 1997 to April 2002. This case-control study has been described previously in detail.[1] Briefly: from the initial case-control study that included 250 patients and 250 controls, we obtained plasma for anti-PS analysis from 244 patients (108 males, 136 females) and 246 controls (107 males, 139 females). The patients were included in the study if they had suffered their first thrombotic event when under 70 years of age. Patients' clinical characteristics are shown in Table 1. The control subjects were recruited according to the following criteria: similar age (±10 years), same sex, no genetic relationship to the patients and no personal or family history of VT. All procedures were approved by the Institutional Review Board of the Hospital de la Santa Creu i Sant Pau in Barcelona. Written informed consent was obtained from all participants.

Blood samples were obtained at least six months after the most recent thombotic event. IgG and IgM anti-PS antibodies were determined by an ELISA kit from Hyphen BioMed (Neuville-sur-Oise), specific for the IgG or IgM isotype. Normal range ≤8.2 AU for IgM and ≤8.9 AU for IgG (99th percentile of the distribution in the control population). The variation coefficient according to the manufacturer ranged from 3 to 6% for intra-assay and 4 to 8% for inter-assay. Tests for LA used Russell viper venom, (Life Diagnostics Frenchs Forest NSW Australia). IgG and IgM APA, including anticardiolipin, antiphosphatidylserine and anti-β2 Glycoprotein I were determined by ELISA methods. Antithrombin, PC, activated protein C resistance, total and free PS, Factor VIII clotting activity, Factor V Leiden (FVL), PT20210A of prothrombin gene, and *F12C46T* polymorphism were analyzed as previously described.[1] Age was expressed as mean ± standard error (SE). The frequencies of variables are expressed in percentages and were compared with the χ^2 test. A logistic regression method was used to estimate both the crude and adjusted Odds Ratio (OR) as a measure of risk. Adjustments were made for sex, age, LA and for other APA as covariables. Also, adjustments were made for those factors previously associated with VT in our population including: FVL, PT20210A, *F12C46T* (*T/T*), and levels of FVIII >90th percentile. The SPSS 14.0 software package was used for statistical analyses.

We found that 12 patients (4.9%) and 2 controls (0.8%) had IgM anti-PS antibodies. The associated thrombotic risk was 6.3 (95% CI: 1.4-28.5). After including LA and APA as covariables, the adjusted OR was 6.1 (95% CI: 1.3-28.0) (Table 2). The risk did not change when other thrombotic risk factors were included in the analysis (adjusted OR: 7.0; 95% CI: 1.5-33.5). After excluding patients with recurrent thrombosis, we studied 183 patients; 8 of them (4.4%) had IgM anti-PS. The associated risk was similar;