chronic warfarin therapy. All three patients were investigated for thrombophilia and no underlying cause was found. The *VHL* core haplotype that has been observed in CP patients,[8] defined by six single nucleotide polymorphisms that span the 137.5 kb of the *VHL* gene, was found in all these three patients.

Patient #5 is a Portuguese girl with a normal P_{50}, normal Epo level and no evidence of cardiac, renal, brain, or adrenal pathology who has polycythemia and ataxia-telangiectasia (A-T). She is heterozygous for a novel *523 A→G (Y175C) VHL* mutation. This mutation is not in the Universal VHL-mutation Database (*www.umd.necker.fra*); however, a mutation in codon 175 was reported in a Spanish patient with pheochromocytoma; no nucleotide details were cited and no further details are available.[9] The parents of our patient are hematologically normal and there was no history of consanguinity. The *VHL* mutation was inherited from her father. The *VHL* gene of her mother was screened for aberrant mRNA transcripts by reverse transcription polymerase chain reactions and the exons and exon/intron boundaries were sequenced in both orientations from genomic DNA, and no mutation was found. A *VHL* null allele (or deletion) in the maternal gene was ruled out since equal proportions of wild-type and mutated nucleotides at 523 (A and G) were found in *VHL* cDNA. We have also considered that the polycythemic phenotype observed in A-T patient #5 may have been caused by a decreased amount of *VHL* mRNA transcript, perhaps caused by nonsense-mediated decay (NMD) secondary to the ATM defect, since the phosphorylation of NMD protein Upf1, a smg2 homolog, is reported to be ATM-dependent.[10] However, as we show in Figure 1, this possibility was ruled out. In summary, we conclude that defects in both alleles of the *VHL* gene may represent the most frequent inherited genetic polycythemic defect; we report two novel *VHL* mutations associated with polycythemia. The molecular biology of the polycythemic patients with a single mutated *VHL* allele remains obscure.

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Key words: congenital polycythemia, VHL mutations, ataxia-telangiectasia.

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Red Cell Disorders

Sustained response to interferon α-2a in thalassemic patients with chronic hepatitis C. A prospective 8-year follow-up study

Eighty-nine thalassemic patients with chronic hepatitis C were treated with interferon α-2a for 12 months and followed up for 8 years. Interferon induced sustained virologic and biochemical response in 45% of participants and histologic improvement in 50% of patients who had paired liver biopsies. Splenectomy was the only independent predictor of an unfavorable outcome.

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Several studies on chronic hepatitis C in thalassemic patients have shown response rates to interferon higher than those in non-thalassemics.[1,2,3-7] These somewhat surprising findings are based on small number of patients with no long-term follow up. Herein, we present the results of a prospective 8-year follow-up study on the efficacy and safety of interferon α-2a in the treatment of chronic hepatitis C in 89 patients with β-thalassemia major.

An open prospective study was conducted from December 1994 through December 1995 to determine the efficacy of interferon α-2a in the treatment of chronic hepatitis C virus (HCV) infection in patients with β-thalassemia major. The participants were recruited from a cohort of 367 thalassemic patients who are followed up at our institution. Eligible patients were those who had detectable HCV RNA in serum, histologic findings consistent with chronic hepatitis, and elevated values of serum alanine aminotransferase. Patients with cardiovascular, endocrine, renal and autoimmune diseases or cirrhosis were excluded from the study as were those who had received prior treatment against hepatitis C. All patients were seronegative for human immunodeficiency virus and immune against hepatitis B virus. The participants were given 3MU of interferon α-2a (Roferon, Roche) subcutaneously thrice weekly for 52 weeks.

At study entry, all participants underwent a physical

Table 1. Virologic biochemical and histologic responses.

Variable	Responders No. of participants	% of responders
Virologic response		
End of treatment	49/89	55
2 years post-treatment	46/89	52
8 years post-treatment	42/80*	53
Biochemical response		
End of treatment	48/89	54
2 years post-treatment	45/89	51
8 years post-treatment	41/80*	51
Combined virologic and biochemical response		
End of treatment	43/89	48
2 years post-treatment	40/89	45
8 years post-treatment	36/80*	45
Histologic response		
End of treatment	24/48	50

**Five patients (one responder and four non-responders) were lost to follow-up and four died (three responders and one non-responder).*

Table 2. Univariate analysis of baseline variables associated with virologic response at the end of treatment.

Variable	Responders n=49	Non-responders n=40	p
Age, years	18.73±0.6	21.10±0.7	0.01
Sex, males/females	30/19	17/23	NS
Splenectomy	4	17	<0.001
Estimated duration of HEP, yrs	5.10±0.46	6.65±0.54	0.03
ALT, IU/dL	199±21	180±16	NS
Serum ferritin, ng/dL	3165±207	3227±230	NS
HCV Genotype			
1b, n	6	7	NS
Other, n	10	12	NS
Liver biopsy			
Hemosiderosis	2.22±0.14	2.56±0.18	NS
Inflammation	5.52±0.35	4.74±0.27	0.09
Fibrosis	2.96±0.20	3.39±0.22	NS

ALT; alanine aminotransferase; HEP: hepatitis.

examination and baseline laboratory testing. Laboratory tests were performed monthly during therapy and every three months during the follow-up period. Serum HCV RNA was determined before initiation of therapy, at the end of therapy, and twice a year during the follow-up period. Anti-HCV was tested by enzyme immunoassay (EIA 2, Abbott Laboratories, Chicago, IL, USA) and confirmed by immunoblotting (Ortho Diagnostics, Raritan, NJ, USA). Serum HCV-RNA was detected by polymerase chain reaction (Amplicor, Roche Diagnostics, NJ, USA). HCV was genotyped using the INNO-LiPA II probe assay (Innogenetics N.V., Belgium) and classified as reported previously.[8] Liver biopsies were performed before enrollment into the study and at the end of treatment. The degree of hepatic inflammation and fibrosis was graded according to the modified Histology Activity Index, proposed by Ishak *et al.*[9] The iron overload in parenchyma and mesenchymal sites was graded by a common 0-4+ practical scheme taking into account the ease of observation and the magnification required. Assessment of treatment efficacy was based primarily on clearance of viremia and secondly on biochemical response and histologic improvement. Virologic response was defined as absence of HCV-RNA and non-response as presence of HCV-RNA in serum at the end of therapy. Relapse was defined as clearance of viremia at the end of treatment followed by reappearance of HCV-RNA during the follow-up period. Biochemical response was defined as normalization or fluctuation of alanine aminotransferase up to two times the normal value. Histologic improvement was defined as a decrease of at least 3 points in the inflammation score from the score of the pretreatment biopsy. Comparisons were made for a range of variables at the end of treatment.

Of 367 thalassemic patients followed up in our institution, 174 were anti-HCV positive, and of these 115 had detectable HCV RNA in their serum. Eighty-nine of these 115 patients were enrolled in the study and 26 were excluded, because they did not meet all inclusion criteria. Males outnumbered females (47/42), the mean age was 19.8±0.46 years (range, 11 to 30 years) and the mean time of alanine aminotransferase elevation before initiation of treatment, was 5.8±0.36 years (estimated duration of hepatitis). Twenty-one individuals had undergone splenectomy before enrollment in the study. Liver biopsy revealed siderosis in all patients, (mean grade 2.4±0.14). The grade of inflammation and score of fibrosis were 5.7±0.30 and 2.9±0.20 points, respectively. HCV genotypes were determined in 35 participants: 13 were infected by genotype 1b and 22 by non-1b genotypes (type 1a, 4; type 3a, 9; 1a/1b, 1; 2a/2c, 4; 4c/4d, 2; 2a/2c/2b, 2).

All participants completed the intended scheduled treatment. The virologic, biochemical and histologic responses are shown in Table 1. Post-treatment liver biopsy specimens were available from 48 patients. A decrease in hepatic inflammation of at least three points was noted in 24 of 48 patients with paired liver biopsies. Of 24 patients with histologic improvement, 18 also had a virologic response and 16 combined virologic and biochemical responses. Treatment had no effect on fibrosis. Variables associated with virologic response are shown in Table 2. Younger patients, not splenectomized ones, and patients with shorter duration of the disease were more likely to respond to interferon treatment. Multiple logistic regression analysis revealed that splenectomy was the only independent factor associated with failure to respond to treatment (odds ratio, 10.19; 95 % confidence interval, 1.97 to 52.69; p=0.006). The adverse events were typical of those produced by interferon. Interferon had to be discontinued temporarily in one patient due to a decrease of hemoglobin requiring additional transfusion.

In this study interferon monotherapy induced sustained virologic responses in approximately half of thalassemic patients with chronic hepatitis C. Clearance of viremia was associated with decreased hepatic inflammation as shown by the reduction of alanine aminotransferase values and histologic improvement on liver biopsies. Since progression of fibrosis to cirrhosis is a function of hepatic inflammation, by reducing inflammation with interferon,

further progression of liver disease in this population of patients may be prevented.

The factors that result in greater therapeutic benefit of interferon in thalassemic patients than in non-thalassemic ones are not known. The young age of thalassemic patients and the short duration of hepatitis may play important roles in the treatment outcome. Alternatively, the multiple blood transfusions required by thalassemic patients may have immunomodulatory effects or other biological properties that enhance the therapeutic effect of interferon. Although several factors were identified as predictors of an unfavorable outcome in the present study, only splenectomy remained as an independent predictor in multivariate analysis. Since splenectomy is performed in patients with increased tranfusional requirements, it is possible that the statistical association of splenectomy with adverse treatment outcome reflects advanced disease with iron overload. These results constitute a challenge to the current treatment of hepatitis C with combined pegylated interferon and ribavirin. Since there is a great concern about combination treatment in thalassemic patients due to ribavirin-induced hemolysis,[10] interferon monotherapy remains the front-line treatment in such patients.

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Chronic Myeloid Leukemia

Factors predicting molecular and cytogenetic response in chronic myeloid leukemia patients treated with imatinib

We studied 94 clinically heterogeneous chronic myeloid leukemia (CML) patients and found that the duration of treatment with interferon-α (IFN-α) prior to imatinib therapy may not improve response to imatinib for patients in chronic phase but a shorter period between CML diagnosis and the initiation of imatinib is predictive for a better molecular response to therapy (p<0.05).

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Ninety-four patients with chronic myeloid leukemia (CML) were analyzed, including 81 enrolled in 4 multicenter international phase 2 or 3 trials (i.e. protocols: 106 *de novo* chronic phase CML (n=11); 110-INF-α failure or resistant or refractory chronic phase CML (n=3); 113-failure or resistant or refractory CML patients (n=52) and 114-accelerated phase CML (n=15)). At the end of the study, 77 patients were in chronic phase of CML and 17 patients were in accelerated phase of the disease. The median follow-up was 45 months (range 12-205). The characteristics of the patients are summarized in Table 1. All patients were treated by imatinib alone; the starting dosage was 400 mg/day for chronic phase patients and 600 mg/day for advanced stage patients. The actual dosage varied in some patients over the time period covered by the present study (300-800 mg/day), depending on tolerance of and/or response to the imatinib therapy. The median duration of imatinib treatment was 17 months (range 4-38).

Predictive factors for response to imatinib therapy in CML patients were estimated after sequential analysis by quantitative reverse transcription polymerase chain reaction (RQ-PCR) on blood samples (relative reduction of bcr-abl mRNA transcript initial values) and conventional cytogenetic monitoring on bone marrow cells. Complete cytogenetic response was defined as 0% Ph-positive metaphases and no cytogenetic response was defined as more than 96% Ph-positive metaphases. For statistical analysis, cytogenetic and molecular responses were analyzed as continuous variables or as categorical variables (i.e complete cytogenetic response or no cytogenetic response and < 2 logs or ≥2 logs, respectively) at 3, 6, 9, 12 and 18 months after the onset of imatinib treatment. Comparisons between groups and relationships between responses and clinical features (sex, age, Sokal score, duration of INF-α therapy, time to imatinib or diagnosis) were tested. RQ-PCR follow-up of bcr-abl mRNA transcript level and conventional cytogenetic response over 6 to more than 12 months revealed that in spite of a wide range of bcr-abl transcript levels observed in patients with complete cytogenetic response and an overlap between each cytogenetic response group, there was linear correlation (p<10^{-4}). For patients in chronic phase CML when starting imatinib, a decrease in Ph-positive metaphase is strongly associated with a decrease in bcr-abl transcript level (p<0.001 after 3, 6, 9 and 12 months of follow-up, and p=0.001 after 18 months of follow-up; Spearman's rank tests). A decrease of more than 2 logs in