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

Table 1. Patients' characteristics.

Characteristics	*Chronic phase CML*	*Accelerated phase CML*
Sex, n. (%)		
Males	47 (61)	11 (65)
Females	30 (39)	6 (39)
Age at diagnosis		
Median, years	50	53
Range, years	17-75	28 -68
Age at onset imatinib		
Median, years	53	59
Range, years	19-78	40-70
Sokal risk group, n. (%)		
Total evaluated	41 (53)	11 (64)
Low	28 (68)	4 (37)
Intermediate	10 (25)	2 (18)
High	3 (7)	5 (45)
Reason for onset imatinib, n.		
Randomization	5 pts	0 pts
INF-α resistance	20 pts	10 pts
INF-α refractory	5 pts	2 pts
INF-α intolerance	29 pts	0 pts
INF-α resistance and intolerance	9 pts	3 pts
INF-α refractory and intolerance	9 pts	2 pts

Table 2. Relationships between clinical features and log reduction of bcr-abl (≤2 or >2).

Clinical features	*Chronic phase of CML*	*Accelerated phase of CML*
Age at diagnosis	NS	NS
Age at onset of imatinib	NS	NS
Sex	NS	NS
Sokal score	NS	NS
Duration of IFN therapy	NS	Longer time allowed, $p< 0.01$
Time to onset imatinib	Shorter time allowed $p<0.05$ at M12	Shorter time allowed $p<0.05$ at M12

47 assessable chronic phase CML patients 3 months after the onset of imatinib or in 19 assessable patients after 6 months of imatinib had significant prognostic relevance for complete cytogenetic response at 12 months ($p<0.05$ or $p<0.01$ by Fisher's exact test, respectively). This observation, consistent with findings in several studies on this subject, confirms that individual RQ-PCR kinetics rather than RQ-PCR continuous values is pertinent for predicting response to imatinib treatment. Data from statistical analyses of relationships between patients' characteristics and bcr-abl log reductions are presented in Table 2. For the 77 patients with chronic phase of CML, a shorter time between diagnosis of CML and the start of imatinib was significantly associated with a greater reduction in bcr-abl transcript level after 6 and 12 months of imatinib therapy ($p<0.05$, Spearman's correlations). Likewise, earlier initiation of imatinib after the diagnosis of CML was significantly related to a more than 2 log decrease after 12 months of treatment ($p<0.05$, Wilcoxon's rank sum test). However, the heterogeneous recruitment and the low number of patients in accelerated phase when starting imatinib leads to inconsistent prediction of response in this group. Of note, we found a borderline significance towards better RQ-PCR molecular response at 9 and 12 months after the start of imatinib in patients with a lower Sokal score (wich is widely accepted as a good predictive factor for patients who are receiving INF-α therapy, and which may also be a good predictive factor of molecular response to imatinib (*data not shown*).

Among clinical features positively related with cytogenetic response after 6 months of imatinib therapy, low initial transcript level, less advanced disease and shorter time from diagnosis were described recently.[1-4] Accordingly, in advanced-stage CML patients, a high incidence of resistance or relapse under imatinib has been demonstrated, suggesting that imatinib may have greater efficiency in recently diagnosed CML patients.[5-8] We describe here that a shorter time allowed between CML diagnosis and initiation of imatinib treatment is a predictive factor for better molecular response to therapy in chronic phase patients and that INF-α pretreatment duration may not improve this response.

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Key words: imatinib, chronic myeloid leukemia, RQ-PCR kinetics, conventional cytogenetics, clinical predictive factors.

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Chronic Myeloproliferative Disorders

Aberrant expression of platelet-derived growth factor (PDGF) and PDGF receptor-α is associated with advanced bone marrow fibrosis in idiopathic myelofibrosis

The expression of members of the platelet-derived growth factor (PDGF) system in bone marrow cells derived from Idiopathic myelofibrosis (IMF) has been investigated by real-time RT-PCR. Increased expression of PDGFs and the corresponding PDGF receptor α could be demonstrated to be a feature of advanced fibrosis in IMF that is not demonstrable in the prefibrotic phase of the disease.

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The platelet-derived growth factor (PDGF) system of PDGF ligands and receptors is thought to play an important role in the fibrogenic process in idiopathic myelofibrosis (IMF). We quantitatively analyzed PDGF isoforms -A, -B, and -C and PDGF receptor α in prefibrotic, cellular IMF, advanced IMF with myelofibrosis, and non-neoplastic hematopoiesis. The 3 PDGF isoforms and PDGF receptor α were significantly overexpressed in advanced IMF with myelofibrosis. We conclude that overexpression of the PDGF system is a pathogenetic feature of advanced myelofibrosis in IMF.

According to the WHO classification, idiopathic myelofibrosis (IMF) refers to a group of chronic myeloproliferative disorders with currently unknown underlying pathogenesis.[1] It is generally accepted that over time prefibrotic, cellular IMF progresses to an advanced stage and bone marrow fibrosis develops. It is also accepted that the proliferation of fibroblasts in bone marrow fibrosis is a reactive rather than a clonal process.[1] The platelet-derived growth factor (PDGF) system of ligands and receptors is widely expressed by a variety of cells and tissues in both physiological and pathological conditions.[2] Among its diverse functions PDGF is known to mediate strong mitogenic signals via PDGF receptors on fibroblasts, endothelial cells, and vascular smooth muscle cells.[2,3] The analysis of PDGF gene expression by bone marrow cells in patients with severe myelofibrosis has so far often been hampered by the inability to collect these cells (*dry tap*). This is a plausible reason for why previous expression studies investigated the PDGF system mainly in peripheral cells. In such studies the levels of PDGF in platelets and plasma derived from IMF patients were found to be elevated.[4] Given that the rate of progression and interval to myelofibrosis are very variable in IMF,[5,6] enhanced PDGF expression could identify cases with an increased risk of progression from the cellular to the fibrotic phase of IMF. On the other hand, PDGF could also be substantially involved in the sustainment of myelofibrosis. In order to investigate potentially aberrant

Table 1. The gene expression level of PDGF isoforms –A, -B, -C, and PDGF-R · in cellular IMF, advanced IMF, and in the control group are illustrated as the median followed by the range (in parenthesis).

	Cellular IMF	*Advanced IMF*	*Control*
PDGF-A	1.1 (0.3-2.6)	2.5 (0.4-8.3)	0.8 (0.2-1.8)
PDGF-B	1.4 (0.2-5.0)	4.4 (1.4-15.8)	1.1 (0.1-3.6)
PDGF-C	1.2 (0.4-3.2)	1.5 (0.4-10.1)	0.9 (0.4-1.6)
PDGF-Rα	1.2 (0.4-3.0)	5.7 (0.4-32.0)	1.1 (0.2-2.2)

The gene expression levels of PDGF isoforms -A, -B, -C, and PDGF-Rα in cellular IMF, advanced IMF, and in the control group are presented as the median followed by the range (in parentheses).

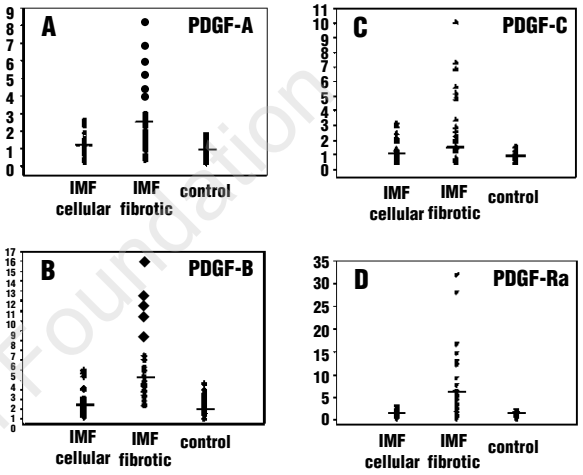

Figure 1. Almost similar PCR efficiencies and successful validation of PCR linearity for PDGF-A (forward 5'-tcgatgagatggagggtcg-3', reverse 5'-acccggacagaaatccagtct-3', probe 5' FAM-cgtgggatg-gaagtgcagaggtctca-TAMRA-3', [9]), PDGF-B (forward 5'-ttcct-gtctctctctgctgcta-3', reverse 5'-atcatcaaaggagcggatcgag-3', probe 5' FAM-cccattcccgaggagctttatgagatgc-TAMRA 3'), PDGF-C (forward 5'-ggagcaccatgaggagtgtga-3', reverse 5'-gagctgctggtggtgatgc-3', probe 5' FAM-tgtgtgcagagggagcacaggaggata-TAMRA 3', [9]), PDGF-Rα (forward 5'-ttcccttggtggcaccc-3', reverse 5'-ggtacc-cactcttgatcttattgtagaa-3', probe 5'FAM-taccccggcatgatggtggattc-tac-TAMRA 3', [9]), β-glucuronidase (β-GUS, forward 5'-ctcatttg-gaattttgccgatt-3', reverse 5'-ccgagtgagatccccctttta-3', and probe 5' FAM-tgaacagtcaccgacgagagtgctgg-TAMRA 3'), and Heat shock protein-70.1 (HSP-70.1, forward 5'-ccggtggtgcagtcgg-3', reverse 5'-ggcttgtctccgtcggttga-3', and probe 5'FAM-catgaagcactggc-ctttccaggtg-TAMRA 3') over a broad concentration range could be demonstrated and enabled quantification relative to the housekeeping genes β-GUS and HSP-70.1 as described elsewhere.[7] Cases of advanced IMF overexpressed all investigated members of the PDGF system as shown in Table 1. Note that horizontal bars represent the median values.