References

1. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982;51:189-99.
2. Sanz GF, Sanz MA, Greenberg PL. Prognostic factors and scoring systems in myelodysplastic syndromes. Haematologica 1998;83:358-68.
3. Aul C, Giagounidis A, Germing U, Ganser A. Evaluating the prognosis of patients with myelodysplastic syndromes. Ann Haematol 2002;81:485-97.
4. Onida F, Kantarjian HM, Smith TL, Ball G, Keating MJ, Estey EH,, et al. Prognostic factors and scoring systems in chronic myelomonocytic leukemia: a retrospective analysis of 213 patients. Blood 2002; 99:840-9.
5. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, et al. World Health Organization Classification of neoplastic diseases of the hemopoietic and lymphoid tissues: report of the clinical advisory committee meeting. J Clin Oncol 1999; 17: 3835-49.
6. Gonzàlez-Medina I, Bueno J, Torrequebrada A, Lopez A, Vallespì T, Massagué I. Two groups of chronic myelomonocytic leucemia: myelodysplastic and myeloproliferative. Prognostic implications in a series of a single center. Leuk Research 2002;26:821-4.
7. Germing U, Gattermann N, Minning H, Heyll A, Aul C. Problems in the classification of CMML-dysplastic versus proliferative type. Leuk Research 1998;22:871-8.
8. Noesslinger T, Reisner R, Gruner H, Tuchler H, Nowotny H, Pittermann E. Dysplastic versus proliferative CMML. A retrospective analysis of 91 patients from a single institution. Leuk Research 2001;25:741-7.
9. Voglovà J, Chrobak L, Neuwirtovà R, Malaskovà V, Straka L. Myelodysplastic and myeloproliferative type of chronic myelomonocytic leukaemia: distinct subgroups or two stages of the same disease? Leuk Res 2001;25:493-9.
10. Germing U, Strupp C, Aivado M, Gattermann N. New prognostic parameters for CMML? Blood 2002;100:731-2.

Chronic Myeloid Leukemia

TP53 codon 72 polymorphism in patients with chronic myeloid leukemia

A single nucleotide polymorphism at TP53 codon 72 means that two alleles exist: A1 (proline residue, Pro72) and A2 (arginine residue, Arg72). The Pro72 variant of p53 has a lower apoptotic potential. We found that allele A1 was more frequent in patients with chronic myeloid leukemia (CML) than in controls, and among CML patients who had no cytogenetic response than among responders.

haematologica 2004; 89:868-869
(*http://www.haematologica.org/2004/7/868)*

Treatment of CML has recently been revolutionized by the introduction of imatinib mesylate, an inhibitor of the *ABL* tyrosine kinase domain.[1] Acquired resistance to imatinib, defined as a relapse following an initial response, occurs and is often characterized by either *BCR-ABL* gene amplification or point mutations in the kinase domain of the protein. These latter cause amino-acid substitutions and prevent imatinib-induced inhibition of $p210^{BCR/ABL}$.[2] Some CML patients fail to obtain a cytogenetic response to initial treatment with imatinib, a condition that is sometimes described as refractoriness or primary resistance to imatinib and whose mechanisms are unknown.[3]

The *TP53* gene, which encodes the p53 tumor-suppressor protein, is characterized by a single nucleotide polymorphism at codon 72, which results in either proline (Pro72, allele A1) or arginine (Arg72, allele A2) at amino-acid position 72. The Pro72 variant of the p53 protein has a markedly reduced capacity to induce apoptosis.[4]

In a study aimed at identifying factors potentially related to imatinib resistance in CML, we analyzed *TP53* codon 72 polymorphism in CML patients. Peripheral blood samples were obtained from 44 patients with chronic phase CML undergoing routine hematologic evaluation at the Division of Hematology, University of Pavia Medical School and IRCCS Policlinico San Matteo, Pavia, Italy, before starting treatment with imatinib. All these 44 CML patients were treated with imatinib mesylate for at least six months before being classified as either responsive or unresponsive to imatinib (Table 1). Further blood samples were taken from the 10 patients who failed to show a cytogenetic response to therapy, as defined by Druker and co-workers,[1] following their diagnosis of cytogenetic refractoriness. In order to study a large number of CML patients, archive samples from a further 52 individuals in chronic phase who had been followed at our Institution in the past were also analyzed. To determine the prevalence of *TP53* A1 and A2 alleles in a population of similar ethnic composition, 174 umbilical cord blood samples were examined.

RNA samples from patients with cytogenetic resistance to imatinib were analyzed for the presence of mutations in the tyrosine kinase domain encoding region of *BCR-ABL* by means of reverse transcription polymerase chain reaction (RT-PCR) and sequencing of PCR products. Codon 72 polymorphism of *TP53* was analyzed by PCR of genomic DNA and subsequent digestion with the restriction enzyme BstUI.[5]

Table 1. Features of CML patients treated with imatinib classified according to cytogenetic response to treatment

Characteristic	*Cytogenetic responders (n=34)*	*Non responders (n=10)*	*Significance*
Age			
Median, *yr.*	46	54	NS
Range, *yr.*	24-70	29-71	
Sex			
Female, no.	12	5	NS
Male, no.	22	5	
Time since diagnosis			
Median, mo.	6	15	NS.
Range, mo.	1-120	1-156	
Sokal risk group			
High, no.	2	4	*P*=0.012
Intermediate, no.	10	0	
Low, no.	14	4	
Data missing, no.	8	2	
BCR/ABL mutations, no.	ND	0	

ND: not determined; NS: not significant.

Table 2. TP53 codon 72 genotypes in control subjects and CML patients.

Subjects (no.)	*A1/A1 no.*	*A1/A2 no.*	*A2/A2 no.*	*A1 allele frequency (%)*
Controls (174)	7	61	106	21.6
CML				
All patients (96)	10	37	49	29.7
Responders* (34)	3	10	21	23.5
Non-responders* (10)	3	5	2	55.0
Archive samples (52)	4	22	26	28.8
Sokal risk group				
High (6)	1	4	1	50.0
Intermediate (10)	0	2	8	10.0
Low (18)	2	6	10	27.8

**Among CML patients, responders and non-responders were classified according to cytogenetic response to imatinib as defined by Druker and co-workers.*[1]

Table 1 summarizes the clinical features of the CML patients treated with imatinib. Ten out of the 44 patients had no cytogenetic response to imatinib, and 2 of them also failed to show hematologic responses. Sequencing of the kinase domain of *BCR-ABL* following documentation of cytogenetic resistance did not identify mutations in these 10 imatinib-refractory patients.

Analysis of *TP53* codon 72 polymorphism (Table 2) showed that allele A1 was more frequent in 96 CML patients than in 174 controls (29.7% vs. 21.6%, respectively; chi square 4.43, p=0.035). This difference was mainly due to an increased proportion of A1 homozygotes among CML patients (10.4% vs. 4.0%, respectively; χ^2 4.43, p=0.038).

When CML patients were analyzed according to selection criteria (Table 2), it was found that the frequency of allele A1 was 28.8% in 52 unselected subjects, 23.5% in 34 patients responsive to imatinib, and 55.0% among patients with cytogenetic resistance to imatinib (χ^2 4.43, p=0.025). Thus, *TP53* A1 allele frequency was higher in this last group than in responsive patients (p=0.007) or in unselected subjects (p=0.023).

The frequency of the A1 allele was also significantly higher among the high Sokal risk group (8/14) than among the low/intermediate Sokal groups (12/56, p=0.016). Of 8 patients with a high Sokal score and/or the A1A1 genotype, 5 (62.5%) had no cytogenetic response to imatinib, whereas only 3 out of the 26 patients (11.5%) in the low/intermediate Sokal groups who had at least one A2 allele were refractory (p=0.005).

These findings suggest that the *TP53* A1 allele may represent a risk factor for both development of CML and primary cytogenetic resistance to treatment with imatinib. Leukemic cells expressing the Pro72 protein may be more resistant to the apoptosis induced by imatinib than cells expressing exclusively the Arg72 protein. Although these cells' proliferation can be modulated by imatinib,[6] they are more likely to survive and cause persistence of a Ph-positive hematopoiesis that may subsequently undergo clonal evolution.[7]

Lack of cytogenetic response is an adverse prognostic factor for hematologic relapse in CML patients treated with imatinib[8] and appears to be risk-related.[9]

Genotyping for the *TP53* codon 72 polymorphism might allow a better definition of risk in the individual CML patient and thus a risk-adapted approach to treatment. For instance, individuals who are homozygous for the *TP53* A1 allele might benefit from high doses of imatinib[10] or alternative therapeutic options at clinical onset.

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Funding: this study was supported by a grants from the Associazione Italiana per la Ricerca sul Cancro (AIRC, Research Project entitled "Functional and molecular profiling of hematopoietic stem cells in myelodysplastic syndromes"), Milan, and from IRCCS Policlinico San Matteo, Pavia, Italy.

Key words: chronic myeloid leukemia, imatinib mesylate, TP53, polymorphism.

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References

1. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001; 344:1031-7.
2. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001; 293:876-80.
3. Branford S, Rudzki Z, Walsh S, Grigg A, Arthur C, Taylor K, et al. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood 2002; 99:3472-5.
4. Dumont P, Leu JI, Della Pietra AC, 3rd, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 2003;33:357-65.
5. Beckman G, Birgander R, Sjalander A, Saha N, Holmberg PA, Kivela A, et al. Is p53 polymorphism maintained by natural selection? Hum Hered 1994;44:266-70.
6. Chu S, Holtz M, Gupta M, Bhatia R. BCR/ABL kinase inhibition by imatinib mesylate enhances MAP kinase activity in chronic myelogenous leukemia CD34+ cells. Blood 2004 (in press).
7. Marktel S, Marin D, Foot N, Szydlo R, Bua M, Karadimitris A, et al. Chronic myeloid leukemia in chronic phase responding to imatinib: the occurrence of additional cytogenetic abnormalities predicts disease progression. Haematologica 2003;88:260-7.
8. O'Dwyer ME, Mauro MJ, Blasdel C, Farnsworth M, Kurilik G, Hsieh YC, et al. Clonal evolution and lack of cytogenetic response are adverse prognostic factors for hematologic relapse of chronic phase CML patients treated with imatinib mesylate. Blood 2004; 103:451-5.
9. Rosti G, Trabacchi E, Bassi S, Bonifazi F, de Vivo A, Martinelli G, et al. Risk and early cytogenetic response to imatinib and interferon in chronic myeloid leukemia. Haematologica 2003; 88:256-9.
10. Kantarjian HM, Talpaz M, O'Brien S, Giles F, Garcia-Manero G, Faderl S, et al. Dose escalation of imatinib mesylate can overcome resistance to standard-dose therapy in patients with chronic myelogenous leukemia. Blood 2003;101:473-5.