Chronic Myeloid Leukemia

Fluorescence *in situ* hybridization analysis of minimal residual disease and the relevance of the der(9) deletion in imatinib-treated patients with chronic myeloid leukemia

Forty-six patients with chronic myeloid leukemia receiving imatinib mesylate (39 in chronic phase, one in accelerated phase, and six in blastic crisis), were studied for a 20-62 month follow-up period by cytogenetics and fluorescence *in situ* hybridization using dual-color, dual-fusion *BCR* and *ABL* probes. This approach provided valuable results for disease management of analysis.

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Minimal residual disease analysis, detecting the Philadelphia chromosome and the *BCR-ABL* molecular rearrangement by fluorescence *in situ* hybridization (FISH) or real-time reverse-transcription polymerase chain reaction (RT-PCR), is of relevance for establishing treatment efficacy in patients with chronic myeloid leukemia (CML).[1-3]

FISH analysis allows identification of the *BCR-ABL* fusion gene in both metaphases and interphase nuclei. However, in early FISH studies in CML patients, the detection limit of neoplastic cells was 1-3% mainly due to the structure of probes used which were unable to distinguish incidental overlapping of signals for *ABL* and *BCR* from real *BCR-ABL* fusion signals.[4-5] Moreover, in 10-15% of CML patients having a t(9;22) combined with a partial deletion on der(9) at band q34, which is associated with an adverse outcome, first-generation FISH probes were useless for distinguishing between interphase normal cells and leukemia cells carrying t(9;22) with der(9) deletion.[6] Recently new combinations of FISH probes have been developed which flank the breakpoint regions on both *ABL* and *BCR* genes. These new probes enable detection of *BCR-ABL* rearrangement and der(9) deletions, improving the test sensitivity to 0.3% or 0.6% for the detection of leukemic cells with the standard t(9;22), or with the der(9) deletion-associated t(9;22), respectively[5] (*and personal unpublished data*).

In our series of 46 patients, 20 had received previous conventional therapy, and nine patients had been previously treated with recombinant interferon α (Table 1). The remaining 17 patients received imatinib mesylate at diagnosis. The dose of imatinib was 400 mg/day in patients in chronic phase and 600 mg/day in patients in accelerated phase or blast crisis. Cytogenetic and FISH analyses were performed at 3, 6, 12 months after initiation of imatinib therapy and 6 monthly thereafter. Forty-five of the 46 patients achieved clinical and hematologic remission. The rate of complete+partial remission detect-

Table 1. Clinical, Philadelphia (Ph) chromosome and FISH-*BCR/ABL* data from CML patients at the start of imatinib therapy.

Previous therapy	*Ph*		*BCR/ABL*		
	100%	*10-75%*	*96-100%*	*5-95%*	*<5%*
Chronic phase	**31**	**8**	**19**	**17**	**3**
Ct	13 (1)	2	9 (1)	6	–
Interferon	5 (1)	4	1 (1)	5	3
no	13 (3)	2	9 (4)	6	–
Accelerated phase	**1**	**–**	**–**	**1**	**–**
no	1	–	–	1	–
Blast crisis	**5**	**1**	**6**	**–**	**–**
Ct	4 (1)	1	5 (1)	–	–
no	1	–	1	–	–

Ct: conventional therapy; no: imatinib commenced at diagnosis; (–): patients with der(9) partial deletion. The average duration of disease before commencing imatinib treatment was 13 months (range, 1-88).

Table 2. Follow-up using cytogenetics and FISH in patients who had additional chromosomal anomalies occurring during imatinib treatment in clinical and hematologic remission (patients 1-7), and in patients with derivative chromosome der(9) deletions (patients 8-14).

	Status at IM start	*Additional anomalies*	*Ph%[a]*	*Status[a] duration[b]*	*Anomaly appearance[b,c]*	*Treatment duration[b]*	*Disease duration[b]*
1	BC	+8,+10,+15,+19,+20	95	BC	+3	12	31[d]
2	BC	+der(22)t(9;22)	90	BC	+18	18	28[d]
3	BC	+der(22)t(9;22)	100	BC	+24	29	113[d]
4	CP	+8,i(17),+der(22)t(9;22)	100	CR	+31	38	50
5	CP	+der(22)t(9;22)/+8	95	CR	+12/+24	33	54
6	CP/IFN	+8,+der(22)t(9;22)/-7[e]	25	CR	+18	37	48
7	CP/IFN	+21e	0	CR	+18	36	60
8	BC	+8,+19	97*[f]	BC	+6	12	66[d]
9	CP	–	95*	BC	–	36	37[d]
10	CP	–	2.8*	CR	–	37	37
11	CP	–	6.6*	CR	–	29	30
12	CP	–	45*	CP	–	27	27
13	CP	–	2.5*	CR	–	23	24
14	CP/IFN	–	16*	CR	–	6	7[g]

*BC: blast crisis; CP: chronic phase; IFN: previous interferon therapy; CR: complete clinical and hematologic remission; [a]: at last follow-up; [b]: in months; [c]: appearance from imatinib start point; [d]: patient died; [e]: Ph-negative clone; [f]: two copies of BCR-ABL on ider(22)t(9;22)(q34;q11), resembling double Ph, at the time of starting imatinib; [g]: submitted to bone marrow transplantation after 7 months of imatinib treatment; *: patients with derivative chromosome der(9) deletions. For each sample at least 20 metaphases and 300 nuclei were analyzed. FISH experiments were carried out using dual-color, dual-fusion BCR and ABL probe combination (Qbiogene-Resnova, Italy).*

ed by FISH (CFR or PFR: *BCR-ABL* absent or present in <35% of cells, respectively) resembled that obtained using conventional cytogenetics [CCyR or PCyR: t(9;22) absent or present in <35% of cells, respectively] throughout the follow-up period and was achieved in 78% of patients. Despite overlapping FISH and cytogenetics results, FISH analysis allowed the investigation of all available samples, 21% of which were unsuitable for cytogenetic analysis.

In our study 33% of samples showing a CCyR actually contained ≥2% leukemic cells by FISH analysis. Moreover FISH revealed leukemic cells in 30% of samples not investigated by cytogenetics. These specimens derived from nine patients (31%) who developed FISH relapse, in five cases eventually associated with clinical recurrence of the disease.

CFR achievement within 12 months of treatment was observed in 14 cases and 94% of these patients exhibited no evidence of cytogenetic or clinical relapse in the subsequent year of treatment, similarly to previous reported data using quantitative RT-PCR.[7-8] CML-blast crisis specific anomalies, representing mechanisms of resistance to imatinib,[9] such as trisomy 8, double Ph, and i(17)(q10), were observed during follow up in five cases in association with acceleration of the disease (Table 2, patients 1-5). A Philadelphia-negative clone was observed in two other patients in clinical-hematologic remission (Table 2, patients 6 and 7), further demonstrating the value of treatment monitoring by conventional cytogenetic and FISH analysis.[7-9]

In 7/46 patients (15%) FISH analysis allowed detection of an interstitial microdeletion on chromosome der(9) including the 5'*ABL* gene (Table 2, patients 8-14). Such a microdeletion is associated with early malignant progression and affects interferon treatment and the outcome of bone marrow transplantation.[6,9] Some authors have suggested that imatinib only partially overcomes the adverse prognostic impact of der(9) deletion.[6] More recently no differences were observed in outcome between CML patients with and without der(9) deletion, possibly due to the high-dose imatinib regimen employed (800 mg/day) and the relatively short follow-up period.[10] In the present series, three of seven patients with der(9) deletion had clinical-hematologic relapse and none of the remaining four patients achieved either CCyR or CFR, being only in PCyR/PFR after 27 months of treatment.

In conclusion, although RT-PCR is a very sensitive approach for evaluating minimal residual disease during imatinib treatment,[1,3,7] in our series FISH analysis with dual-color, dual-fusion probe demonstrated its usefulness for CML molecular follow-up. In addition, the present results and those of other groups suggest that microdeletion on chromosome der(9) is a negative prognostic feature also in CML patients treated with imatinib, including those who start this treatment at diagnosis.

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