Persistence of chromosomal abnormalities additional to the Philadelphia chromosome after Philadelphia chromosome disappearance during imatinib therapy for chronic myeloid leukemia

Five Philadelphia chromosome positive (Ph⁺) chronic myeloid leukemia (CML) patients with additional chromosome abnormalities at diagnosis have been followed during Imatinib therapy. In all, the Ph chromosome disappeared, while the 5 cases, additional abnormalities [dup(1); del(5), +8 (2 patients) and +14] persisted in the subsequent studies, performed over a period of 11 to 49 months, either alone or together with a karyotypically normal cell population. This finding is consistent with a secondary origin of the Ph chromosome in these patients. It is still to early to evaluate the possible prognostic value of these additional abnormalities.

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The emergence of chromosome abnormalities in Ph negative (Ph⁻) cells during Imatinib treatment in CML patients has been widely observed although origin and prognostic significance are still uncertain.

However, a few patients carry chromosome changes in addition to the Ph chromosome since diagnosis have been reported.[1-3] In these cases, imatinib treatment induces the disappearance of the Ph⁺ population, allowing the clone marked by the additional abnormality alone to persist. This observation may have important prognostic and pathogenetic implications. Based on some of our initial observations, we contacted the 54 Cytogenetics Laboratories participating in the GIMEMA CML Working Party Group. Out of the 30 laboratories who answered our request, 4 provided 5 patients, who are the subject of this study. Incidence therefore is seen to be under 1%. Three were males and 2 females, median age was 77 years (range 43-80). Two patients had an intermediate and 3 a high Sokal Index. All patients were treated with Imatinib from diagnosis. Sequential chromosome analyses of the 5 patients are shown in Table 1. Additional abnormalities were: dup(1q), del(5q), +8 (two patients) and +14, respectively. All four patients showed the complete disappearance of the Ph-positive clone in a period of time varying from 3 to 16 months, with persistence of the clone carrying the additional abnormality, either alone or together with a karyotypically normal cell population. Of particular interest is patient n. 4 who at diagnosis presented three different clones in addition to the normal cell population: 47,XY,+8; 46,XY,Ph⁺; 47,XY,Ph⁺,+8. The presence of the Ph chromosome both in normal and trisomy 8 cells might indicate a biclonal origin of the Ph. Eight months after diagnosis, the patient developed a Ph⁻ myeloid blast crisis marked by a trisomy 8, already present at diagnosis. PHA-stimulated lymphocytes of all five patients were studied. A normal chromosome pattern was observed in all 100 metaphases analyzed for each patient. These observations may have important pathogenetic implications. The fact that cytogenetic abnormalities present since diagnosis in addition to the Ph chromosome persisted in Ph-negative cells during Imatinib therapy, clearly suggests that they occurred as primary events. Therefore, our study supports the hypothesis that the clones observed in different patients arose from a genetically unstable progenitor cell with a normal karyotype which acquired subsequent cytogenetic abnormalities, of which the Ph chromosome was secondary.

Table 1. Sequential karyotypes of the 5 patients.

Pts. (sex, age)	*Months from diagnosis*	*Karyotype*
1) M.M. (F, 77)	D	46,XX,t(9;22)(q34;q11),dup(1)(q11q21) [20]
	+4	46,XX,dup(1)(q11q21) [20]
	+13	46,XX,dup(1)(q11q21) [20]
	+18	46,XX,dup(1)(q11q21) [20]
	+25	46,XX,dup(1)(q11q21) [20]
	+31	46,XX,dup(1)(q11q21) [20]
	+39	46,XX,dup(1)(q11q21) [20]
	+49	46,XX,dup(1)(q11q21) [20]
2) B.A.) (M, 77	D	46,XY,t(9;22)(q34;q11),del(5)(q14q31) [20]
	+4	46,XY [15]/46,XY,t(9;22)(q34;q11),del(5)(q14q31) [4]/ 46,XY,del(5)(q14q31) [1]
	+9	46,XY,t(9;22)(q34;q11),del(5)(q14q31) [11]/46,XY [9]
	+12	46,XY [10]/46,XY,t(9;22)(q34;q11),del(5)(q14q31)[6]/ 46,XY,del(5)(q14q31) [4]
	+15	46,XY [15]/46,XY,t(9;22)(q34;q11),del(5)(q14q31) [3]/ 46,XY,del(5)(q14q31) [2]
	+18	46,XY [15]/46,XY,del(5)(q14q31) [5]
	+24	46,XY [16]/46,XY,del(5)(q14q31) [4]
	+36	46,XY [15]/46,XY,del(5)(q14q31) [4]
	+42	46,XY [15]/46,XY,del(5)(q14q31) [5]
3) F.D. (M, 65)	D	46,XY,t(9;22)(q34;q11)[16]/47,XY,t(9;22)(q34;q11),+8 [8]/47,XY,+8 [4]/46,XY[2]
	+5	47,XY,+8 [14]/ 46,XY[8]
	+8	47,XY,+8 [22]
	+11	Death in BC
4) B.P. (M, 80)	D	46,XY,t(9;22)(q34;q11),+14 [22]
	+8	47,XY,+14 [15]/46,XY [5]
	+15	47,XY,+14 [16]/46,XY [4]
	+24	47,XY,+14 [14]/46,XY [6]
	+29	47,XY,+14 [17]/46,XY [3]
5) S.S. (F, 43)	D	46,XX,t(9;22)(q34;q11) [5]/47,XX,t(9;22)(q34;q11),+8[4]
	+7	46,XX,t(9;22)(q34;q11) [13]/47,XX,+8 [5]/46,XX [1]
	+11	47,XX,+8 [7]/46,XX [2]

D: diagnosis.

This could eventually be true also for submicroscopic aberrations and the phenomenon could be more frequent than had been believed on the basis of cytogenetic investigations alone.[4]

Moreover, the occurrence of additional chromosome changes may also have a prognostic value. Those observed in our patients are non-specific and are seen, for example, in acute myeloid leukemia, myeloproliferative disorders or myelodysplastic syndromes. One of our patients died (n. 3), while the other 4 are alive and in hematologic and cytogenetic complete remission 49+, 42+, 29+ and 11+ months from diagnosis respectively. The occurrence of additional non-random chromosome abnormalities during the course of the disease has been extensively described in the past and considered an unfavorable prognostic factor, particularly when belonging to the *major route* type.[5,6] However, a few studies deal with the outcome of patients who show additional chromosome changes since diagnosis. A shorter survival was documented in two series belonging to the hydroxyurea/busulfan era.[7,8] In another series including patients treated with α interferon, additional chromosome abnormalities at diagnosis were not associated with a poorer

outcome, with the exception of patients with +8, +Ph and iso(17q).[9] Imatinib treatment has greatly modified the course of Ph-positive CML. Nevertheless, resistance to Imatinib is associated with well-defined chromosome and molecular changes.[10] Information regarding the fate of the subgroup of patients carrying additional chromosome changes since diagnosis is still lacking. The only patient who died in this study was characterized by the persistence of a *major route* change. The follow-up of the other patients, characterized by chromosome abnormalities either included (n.5 with +8) or not in the *major* route type, will be pursued and the cooperative study will continue recruitment of such patients. At the moment, they are all in complete hematologic and cytogenetic response.

Performing classic cytogenetics both at diagnosis and during the course of the disease is still the only way for detecting the presence and/or the development of additional chromosome changes before and during imatinib therapy, with possible pathogenetic, prognostic and, consequently, therapeutic implications.

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