The hypereosinophilic syndrome: fluorescence *in situ* hybridization detects the del(4)(q12)-*FIP1L1/PDGFRA* but not genomic rearrangements of other tyrosine kinases

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Background and Objectives. According to WHO criteria, the idiopathic hypereosinophilic syndrome (HES) is defined as persistent eosinophilia (>1.5×10^9/L) without underlying causes, which is associated with signs or symptoms of organ involvement. Increased bone marrow blasts (>5%) or cytogenetic/genetic markers indicate chronic eosinophilic leukemia (CEL). A cryptic deletion of 4q12, i.e. del(4)(q12), producing the *FIP1L1/PDGFRA* fusion gene, identifies a distinct CEL subgroup (4q-/CEL). Our aims were: a) to use interphase-fluorescent *in situ* hybridization (FISH) to detect the cryptic 4q12 deletion; b) to compare the clinico-hematologic features of 4q-/CEL with other HES; c) to investigate whether *PDGFRB*, *FGFR1*, *ABL1*, and *ETV6*-activated tyrosine kinases are rearranged in CEL/HES.

Design and Methods. This multicenter study included 20 patients fulfilling the WHO criteria for HES and 6 patients without signs/symptoms of end-organ involvement. Double-color FISH was applied in all cases to investigate del(4)(q12). Further interphase-FISH assessed whether *PDGFRB*/5q33, *FGFR1*/8p11, *ABL1*/9q34, and *ETV6*/12p13 undergo rearrangements in HES.

Results. Ten of the 26 patients (9 males and 1 female) had a cryptic del(4)(q12)-*FIP1L1/PDGFRA* which was confirmed by reverse transcription polymerase chain reaction (RT-PCR) analysis in four. Hepatomegaly and splenomegaly were significantly more frequent in these 10 than in the other 16 patients. Seven of these 10 patients received imatinib mesylate therapy and all achieved hematologic remission. In 3 of the patients interphase-FISH and RT-PCR demonstrated cytogenetic and molecular remission. Improvements were observed in signs and symptoms of cardiac and central nervous system involvement in 2 and 1 patient, respectively. Rearrangements of *PDGFRB*, *FGFR1*, *ABL1*, or *ETV6* were not detected in this study.

Interpretation and Conclusions. FISH is a reliable diagnostic test for differentiating 4q-/CEL from other forms of HES, allowing an early diagnosis of good responders to imatinib mesylate therapy. We show that *PDGFRB*, *FGFR1*, *ABL1* and *ETV6* were not rearranged in the HES and 4q-/CEL cases we studied.

Key words: HES, CEL, tyrosine kinases, FISH.

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Tyrosine kinase activation is the common denominator in myeloid malignancies with bone marrow and/or peripheral blood eosinophilia. In fact, increased eosinophils in peripheral blood and/or in bone marrow are associated with the t(5;12)(q33;p13)-*ETV6/PDGFRB* and other *PDGFRB* translocations.[1] In acute eosinophilic leukemia and in several other myeloid and lymphoid diseases with eosinophilia, tyrosine kinases such as ARG/ABL2, JAK2, Syk, and ABL1 are rearranged with the *ETV6* gene and in all these instances, tyrosine-kinase activation depends on the *ETV6* dimerization domain.[2-4]

In an atypical stem cell myeloproliferative disorder, the so-called *8p11 syndrome*, with peripheral blood eosinophilia, B- or T-cell lymphoblast leukemia/lymphoma and rapid progression to acute myeloid leukemia, one transmembrane tyrosine kinase receptor, FGFR1/8p11, is rearranged with different gene/chromosome partners (*FOP*/6q27, *TIF1*/7q34, *CEP110*/9q33, 11p15, *FGFR1OP*/12p11, 12q15, *ZNF*198/13q12, 17q25, *HERV-K*/19q13) promoting its dimerization and autophosphorylation.[5-9] In Philadelphia-negative chronic myeloid leukemia (CML) the *FGFR1* gene is activated in a t(8;22)(p11;q11) *BCR/FGFR1*.[10]

The idiopathic hypereosinophilic syndrome (HES) is characterized by a persistently high eosinophil count (> 1.5×10^9/L), signs or symptoms of organ involvement and no secondary causes such as allergies, atopic diseases and asthma, infections (mainly helminthic), autoimmune disorders, exposure to toxins, solid or hematopoietic neoplasias.[11] Increased blast counts in blood or bone marrow and/or detection

of clonal abnormalities lead to a diagnosis of chronic eosinophilic leukemia (CEL). In a CEL subgroup with strong response to imatinib mesylate therapy, genetic analysis has recently identified a cryptic del(4)(q12), producing the FIP1L1/PDGFRA fusion protein.[12,13] Furthermore in a subgroup of atypical CML with splenomegaly and eosinophilia, the *PDGFRA* gene, encoding a tyrosine-kinase protein, is involved as the *BCR* partner in the t(4;22)(q12;q11).[14]

In this study we used a fluorescent *in situ* hybridization (FISH) approach to check deletion or translocation of the genes involved in different malignant eosinophilic conditions in a series of cases diagnosed as having idiopathic hypereosinophilic syndrome.

Design and Methods

Patients

This study was carried out in 20 patients fulfilling the WHO criteria for the diagnosis of HES and in 6 patients without signs or symptoms of end-organ involvement. Patients were enrolled at the Hematology Departments of the following Italian Universities: Perugia, Florence, Bari, Naples, La Sapienza, Rome, Ferrara and the San Raffaele Hospital, Milan; at the Servei de Hematologia, Hospital Sant Pau, Barcelona, Spain and the Health, Physics and Environmental Hygiene Laboratory, NCSR Demokritos, Athens, Greece.

Cytogenetics

Bone marrow cells were cultured for 24 or 48 hours. Metaphases were G-banded with Wright's stain and karyotypes were described according to the International System for Human Cytogenetic Nomenclature (1995).[15]

Fluorescence in situ hybridization

Interphase-FISH was performed as previously described.[16] BAC 3H20 (mapping between *PDGFRA* and *FIP1L1*) with BAC 120K16 (mapping centromeric to *FIP1L1*) or BAC 24O10 (mapping telomeric to *PDGFRA*) were used in double-color experiments to study the cryptic del(4)(q12)-*FIP1L1/PDGFRA* in all cases.[13] Further FISH investigations were performed with cosmids 4-1 and 9-4 for *PDGFRB/5q33*,[17] BAC 350N15 and/or PAC 162N14 and PAC 224C10 for *FGFR1*/8p11,[18,19] PAC 1132H12, PAC 913J14, and PAC 888H11 for *ABL1*/9q34,[20] and cosmids 179A6 and 148B6 for *ETV6*/12p13[21] in selected cases with available fixed cells. BAC and PAC clones were obtained from the Roswell Park Cancer Institute libraries RPCI-1, RPCI-5, and RPCI-11, *http://www.chori.org/BACPAC*. FISH data were analyzed with a fluorescence microscope (Provis, Olympus). Two hundred nuclei were scored for each probe on samples from diagnosis and 500 nuclei were scored for del(4)(q12) during follow-up. Normal bone marrow samples were added in each experiment as controls. The cut-off limits for monosomy/deletion (5%/del(4)(q12), 6%/*PDGFRB*, 5%/*FGFR1*, 9%/*ABL1*, and 6%/*ETV6*) and trisomy/splitting (1%/del(4)(q12), 2%/*PDGFRB*, 1%/ *FGFR1*, 1.5%/*ABL1*, and 2.5%/*ETV6*) were the highest normal values.

Molecular studies

Nested reverse transcription polymerase chain reaction (RT-PCR) analysis was performed on bone marrow samples at diagnosis in patients 5, 6, 7, and 10 and during imatinib therapy in patients 6, 7, and 10, as previously described.[13]

Results

Tables 1 and 2 show the clinical and hematologic data of the 26 patients. Patient #6 has been reported in detail elsewhere.[22] The 17 males and 9 females (ratio 1.9) had a median age of 51 years (range 10-87). The eosinophil counts ranged between 1.9 and 36.1 mm^3. Leukocytosis was observed in 19 patients (range of WBC: 11×10^9/L to 62.6×10^9/L), anemia in 11 (range of Hb: 8.2-12.8 g/dL) and thrombocytopenia in 5 (range of platelets: 31×10^9/L to 69×10^9/L). Hepatomegaly and splenomegaly were found in 11 and 13 patients, respectively. Signs and/or symptoms of end-organ involvement were present in 20/26 cases: 11 patients had skin lesions, 5 had heart and 5 had central nervous system (CNS) involvement. The lung and gastrointestinal tract were each affected in 4 patients. Seven patients showed simultaneous involvement of two or three organs (pts. #6, 7, 11, 18, 20, 21, 24; see Table 1). Hepatomegaly and splenomegaly were significantly ($p<0.04$ for both) more frequent in 4q-/CEL than in HES. The gastrointestinal tract was involved only in HES (4 patients). Damage to more than one organ was observed in 2/10 patients with 4q-/CEL and in 5/16 HES patients.

Morphological examination of bone marrow aspirates showed increased eosinophil counts that varied greatly in the 20 patients with available data (Table 1). Bone marrow biopsy (BM) was performed in 16 patients: a widespread increase in reticulum was observed in three patients with 4q-/CEL (pts. #5, 6 and 9; see Table 1), and myeloid hyperplasia in eight patients (#3, 7, 8, 9, 10 with 4q-/CEL; #20, 23, and 25 with HES; see Table 1). Myeloid hyperplasia and increased reticulum, with no rise in eosinophil precursors, was observed in two consecutive bone marrow biopsies from patient #9. Bone marrow blast cells were within the normal range (<5%) in all 23 patients for whom morphology and/or histology findings were available. Karyotypes were normal in all the 20 patients

Table 1. Clinical and hematologic findings in 10 patients with 4q-/CEL and 16 with the hypereosinophilic syndrome.

Patient	Age/Sex	WBC $\times 10^9$/L	Eosinophil count (mm^3)	S/H*	End-organ involvement	Bone marrow aspirate/biopsy
			FIP1L1/PDGFRA positive 4q-/CEL			
1	29/M	129	11480	Yes/Yes	Skin	↑ eos precursors/ n.a.
2	48/M	62.6	30050	Yes/Yes	CNS	25% eos/ n.a..
3	34/M	7	3570	Yes/Yes	Skin	70% eos/ myeloid hyperplasia, ↑ eos
4	67/M	32	9600	Yes/Yes	No	15% eos/↑ eos
5	68/F	32.2	4500	Yes/Yes	No	12% eos/↑ eos, ↑ diffuse fibrotic reticulum
6	37/M	22	13900	No/No	Lung/Heart	↑ eos/↑ eos, ↑ diffuse fibrotic reticulum
7	26/M	22.6	12900	Yes/Yes	Skin/CNS	n.a./ myeloid hyperplasia, ↑ eos
8	29/M	16.2	10200	No/No	Heart	61% eos /myeloid hyperplasia, ↑eos
9	62/M	23.5	11280	Yes/No	No	n.a/↑ diffuse fibrotic reticulum, myeloid hyperplasia
10	29/M	34	2100	YesYes	Lung	62% eos/ myeloid hyperplasia
			FIP1L1/PDGFRA negative HES			
11	10/M	11.5	5750	Yes/No	Heart/Skin/CNS	↑ eos/ n.a
12	22/M	24	4320	No/No	No	94% eos/↑ eos
13	55/M	42.6	14910	Yes/Yes	No	n.a./ n.a.
14	39/F	8.5	2125	Yes/Yes	CNS	20% eos/↑ eos
15	87/M	26	16640	No/No	Gastrointestinal tract	38% eos/ n.a.
16	78/F	4.7	1900	No/No	Skin	n.a./ n.a.
17	25/F	5	2450	No/No	Gastrointestinal tract	38% eos/ n.a.
18	60/F	18.8	2250	Yes/Yes	Lung/Skin	34% eos/↑ eos
19	71/F	12.9	7752	No/Yes	Heart	80% eos/ n.a.
20	78/M	21.3	11080	No/No	Lung/CNS/Skin	↑ eos/ myeloid hyperplasia, ↑ eos
21	50/F	8.3	2700	No/No	Skin/ Gastrointestinal tract	24% eos/↑ eos
22	78/F	17.3	7960	No/No	No	↑? eos/ n.a.
23	72/F	11	2860	No/No	Skin	↑ eos/ myeloid hyperplasia, ↑ eos
24	75/M	7.9	1900	No/No	Skin/ Gastrointestinal tract	n.a./ n.a.
25	35/M	42	36100	Yes/No	Heart	60% eos/ myeloid hyperplasia, ↑ eos
26	72/M	7.5	2100	No/No	Skin	n.a./↑ eos

M: male; F: female; WBC, white blood cell; CNS: central nervous system; n.a.,: not available; ↑, increase; eos, eosinophils; S/H, splenomegaly/hepatomegaly are significantly more frequent in 4q-/CEL than in HES (p< 0,04 for both; Fisher's exact test).*

Table 2. Treatment and survival of 10 patients with 4q-/CEL and 16 with the hypereosinophilic syndrome.

Patient	Age/Sex (months)	Treatment	Follow-up
		FIP1L1/PDGFRA positive 4q-/CEL	
1	29/M	Steroids, 1 month HU 2 g/day, 1 month	6 died
2	48/M	Steroids + HU 6 g/day, 30 months Steroids + IFN 3,000,000 UI Steroids + Imatinib 100 mg/day, 12 months	110*
3	34/M	HU, 7 months 1° aBMT 14-09-00; 2° aBMT 9-01-01	45
4	67/M	Unknown	Dropped out
5	68/F	Imatinib 100 mg/day, 9 months	22*
6	37/M	Imatinib 200 mg/day, 2 weeks; 100 mg/day 10 months	12*
7	26/M	Imatinib, 100 mg/day, 22 months	9*
8	29/M	Steroids 5 months Imatinib 100 mg/day	7*
9	62/M	Imatinib 400 mg/day, 1 month	7*
10	29/M	Imatinib 100 mg/day, 14 months	9*
		FIP1L1/PDGFRA negative HES	
11	10/M	Steroids and HU for 3 years, Steroids and IFN or Hu, alternatively	204
12	22/M	Steroids and HU 4 years	168
13	55/M	No	144
14	39/F	Steroids and HU, 7 years Steroids and Imatinib, 3 m	84
15	87/M	Steroids 20 mg/day, 3 years	36
16	78/F	Steroids and HU	48
17	25/F	Imatinib 200 mg/day, 3 weeks Steroids, 3 months	36
18	60/F	Steroids, 29 months	36
19	71/F	Steroids	Dropped out
20	78/M	Steroids, 4 months	23
21	50/F	Steroids and Imatinib 200 mg/day, 9 months	25*
22	78/F	HU, 4 months	18
23	72/F	Steroids, 2 years	8
24	75/M	Steroids and Imatinib 200 mg/day, 7 months	19*
25	35/M	Steroids and Imatinib 600 mg/day, 3 months	13
26	72/M	Steroids, 7 months	11

*M: male; F: female; IFN: α-interferon; HU: hydroxyurea; aBMT: allogeneic matched bone marrow transplantation; *cases with documented hematologic response after imatinib mesylate; steroids, different schedules include a dosage between 5 mg-25 mg/day.*

Table 3. Cytogenetic and FISH findings in 26 patients with the hypereosinophilic syndrome.

Patients	*Age/Sex*	*Karyotype*	*RP11-3H20**	*cosmids 9-4+ 4-1*	*RP11-350N15 and RP1-162N14/RP1-224C10*	*RP5-1132H12/RP-5-913J14/RP5-888H11*	*Cosmids 179A6/148B6*
				FIP1L1/PDGFRA positive 4q-/CEL			
1	29/M	46,XY [15/15]	4	99	n.d.	99	92
2	48/M	46,XY [17/17]	5	97	95	98	96
3	34/M	46,XY [18/18]	33	n.d.	97	96	100
4	67/M	46,XY [20/20]	10	99	98	95.5	96
5	68/F	46,XX [20/20]	15	95.5	99	n.d.	97
6	37/M	46,XY [12/12]	56	95	n.d.	n.d.	n.d.
7	26/M	46,XY [20/20]	22	98	97	98	100
8	29/M	46,XY [20/20]	55	99	99	98.5	99.5
9	62/M	n.a.	17	99	n.d.	n.d.	n.d.
10	29/M	46,XY [20/20]	22	99	100	98	9
				FIP1L1/PDGFRA negative HES			
11	10/M	46,XY [15/15]	99	n.d.	n.d.	n.d.	n.d.
12	22/M	46,XY [20/20]	97	97	100#	94	94
13	55/M	n.a.	99	97	98#	100	99.5
14	39/F	46,XX [20/20]	98	99	98#	99	97
15	87/M	46,XY [27/27]	97	99	100#	95.6	96
16	78/F	n.a.	98.5	100	n.d.	93.5	99
17	25/F	46,XX [20/20]	92	n.d.	96	94	93
18	60/F	46,XX [20/20]	96.5	99	99#	95	98.5
19	71/F	46,XX [15/15]	97	99.5	99.5#	98	99.5
20	78/M	46,XY [15/15]	99.5	96.5	92	93	95
21	50/F	n.a.	97.5	97.5	100#	98	100
22	78/F	46,XX [20/20]	98.5	n.d.	n.d.	n.d.	n.d.
23	72/F	46,XX [15/15]	95	n.d.	n.d.	n.d.	n.d.
24	75/M	n.a.	98	98	97	92	99
25	35/M	46,XY [20/20]	95	97	99	95	94
26	72/M	n.a.	95	96	99#	96	98

*M, male; F, female; n.a., not available; *RP11-3H20 was used with RP11-120K16 or RP11-224O10 as internal control in double-color experiments; cosmid 9-4 for the 3'PDGFRB and cosmid 4-1 for the 5'PDGFRB; RP11-350N15 encompasses the entire FGFR1 gene; #patients studied also with clone RP1-162N14 and RP1-224C10 flanking the 3' and the 5' end of FGFR1, respectively; RP5-1132H12, RP5-913J14, and RP5-888H11 encompass the ABL1 breakpoints; cosmid 179A6 maps at the 5'ETV6 and cosmid 148B6 at the 3'ETV6; numbers indicate the percentage of disomic nuclei for each clone tested; n.d., not done.*

studied (Table 3). In 10/26 patients, FISH showed a cryptic 4q12 deletion with one hybridization signal in 44% to 96% of interphase nuclei using clone RP11-3H20 with either RP11-120K16 or RP11-24O10 as the internal control at diagnosis (Figure 1). A normal hybridization pattern was found in the three cases (pts. #6, 7, and 10) monitored during imatinib mesylate therapy (after 3, 3, and 5 months, respectively). The hybridization patterns of probes for *PDGFRB*, *FGFR1*, *ABL1*, and *ETV6* were normal both in patients with HES and in patients with 4q-/CEL (Table 3).

RT-PCR detected the FIP1L1/PDGFRA fusion protein in patients #5, 6, 7 and 10 at diagnosis. RT-PCR was negative in cases #6, 7, and 10 after respectively 3, 8, and 5 months of imatinib mesylate therapy. Lines of therapy are reported in Table 2. Patients received different treatments, including steroids (17 patients), α-interferon (2 patients), hydroxyurea (8 patients), and imatinib mesylate (12 patients). Patient 1 (4q-/CEL) rapidly progressed to develop refractory acute myeloid leukemia and died within 6 months of diagnosis. Patient #13 was not treated. Patients #4 and 19 dropped out of follow-up. Patient #3 (4q-/CEL) underwent two matched bone marrow transplants and is alive and well after a follow-up of 45 months. The median follow-up of the other 21 cases was 47 months (range 7-204 months). Improvements in end-organ symptoms were observed in three 4q-/CEL patients. In patients #6 and 8 who were suffering from Loeffler's endocarditis, symptoms promptly resolved and transthoracic Doppler echocardiography findings were normal in both soon after starting imatinib therapy.[22] In patient #7, who had diplopia and several hyperdense lesions of the white cerebral matter, neurological symptoms resolved after 11 months of imatinib therapy, and magnetic resonance imaging demonstrated a reduction in all hyperdense lesions.

Discussion

The identification of *FIP1L1/PDGFRA* provided new insights into the treatment of patients with HES/CEL and, in fact, imatinib mesylate therapy has significantly changed the course of this malignancy.[12,13]

In this multicenter study on 26 retrospective cases of

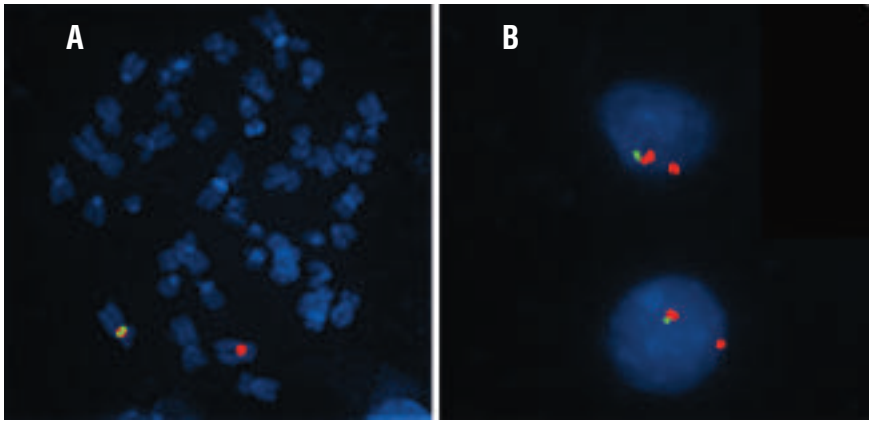

Figure 1. Double-color FISH with clone RP11-3H20 (green) and clone RP11-120K16 (red) in patient 10. **Panel A.** The red/green signal indicates normal chromosome 4; the red signal alone indicates a cryptic deletion on the other chromosome 4 (arrow). **Panel B.** Interphase nuclei with two red signals and one green signal.

primary hypereosinophilia, we used interphase FISH to detect the del(4)(q12)-*FIP1L1/PDGFRA* change and to investigate whether other genes that are rearranged in myeloid malignancies with eosinophilia are involved in HES. Interphase FISH detected cryptic del(4)(q12) in 10/26 patients (#1-10; Table 1) but no rearrangements of *PDGFRB*, *FGFR1*, *ABL1*, or *EVT6*. It is worth noting that known oncogenic tyrosine kinases were not implicated in either *FIP1L1-PDGFRA*-negative or positive cases in this study.

Differences in clinical features emerged between HES and 4q-/CEL, the incidence of which was 38.5% in our series. There was a significantly higher incidence of hepatomegaly and splenomegaly in the 4q-/CEL group (p<0.04 for both; Fisher's exact test). The male/female ratio was 8/8 and 9/1, respectively. Male predominance has already been found in other hematologic disorders associated with rearrangements of either *PDGFRA* or *PDGFRB*, although the causes are still unknown.[1,12]

Interestingly, in 3 cases (#4, 5 and 9) that did not completely satisfy the WHO criteria for either HES or CEL since they did not show end-organ involvement, we found the del(4)(q12)-*FIP1L1/PDGFRA* lesion. Two of these patients were among the seven 4q-/CEL patients (#2, 5, 6, 7, 8, 9, and 10) who were successfully treated with imatinib mesylate, with the peripheral eosinophil count normalizing within 2-4 weeks. Another of the seven was our only female patient (#5) with 4q-/CEL who also achieved complete hematologic remission, confirming another report of a good response in a female patient.[13] In three patients (#6, 7, and 10), interphase FISH and RT-PCR demonstrated cytogenetic and molecular remission during therapy. In the two patients with Loeffler endocarditis (#6 and 8), imatinib mesylate was associated with regression of cardiac symptoms and resolution of transthoracic echocardiography abnormalities.[22] Diplopia regressed and improvements in magnetic resonance imaging were observed in patient #7 with CNS involvement. The efficacy of imatinib mesylate in inducing remission or improvement of signs and symptoms of CNS involvement have already been reported in two patients with 4q-/CEL.[23,24]

Finally, five of 16 patients with HES who were *FIP1L1/PDGFRA*-negative underwent imatinib mesylate treatment. Two patients (#21 and 24) achieved hematologic remission with peripheral eosinophil count normalization, confirming previous observations of succesful imatinib therapy in a subgroup of *FIP1L1/PDGFRA*-negative CEL.[12,25] We are unable to explain the underlying biological response because the genetic lesion in patients without the 4q$^-$/CEL who responded to imatinib mesylate therapy remains to be elucidated. However, this study has proven it is not related to genomic rearrangements of *PDGFRB* and *ABL1* tyrosine kinases or partners of *ETV6*.

In conclusion, in a selected series of patients with primary hypereosinophilic syndrome the incidence of the 4q-/CEL is around 40% with no involvement of the other tyrosine kinases we tested. Prospective multicenter studies are needed to determine the true incidence and treat patients accordingly. As FISH studies diagnosed 4q-/CEL in three cases which did not satisfy the WHO definition of HES/CEL because of absence of end-organ involvement, inclusion criteria for these studies remain an open question.

RLS was the principal investigator. GS, AC, DB, CN, LL, AA, CS, NT, and MF provided clinical and cytogenetic data of patients from their Centers.PM performed molecular studies. MFM was involved in diagnosis and management of patients. CM was responsible for the conception and supervision of the study and in drafting the paper. PAC 162N14 and PAC 224C10 were kindly provided by the Sanger Institute, Oxford, United Kingdom. BAC 350N15, PAC 1132H12, PAC 913J14, and PAC 888H11 were kindly provided by Dr. M. Rocchi, DAPEG, Sez. di Genetica, University of Bari, Italy. The authors wish to thank Dr. Barbara Anaclerico for providing clinical data of patients #21, 24, and 26, Dr. Graziella Gurdo for providing clinical data of patient #7, and Dr. Geraldine Boyd for assistance in the preparation of the manuscript. The authors declare that they have no potential conflict of interest.

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References

1. Steer EJ, Cross NCP. Myeloproliferative disorders with translocations of chromosome 5q31-35: role of the platelet-derived growth factor receptor. Acta Haematol 2002; 107:113-22.
2. Lacronique V, Bourex A, Della Valle V, Poirel H, Tran Quang C, Mauchauffé M, et al. A TEL-JAK2 fusion protein with constitutive kinase activity in human leukemia. Science 1997; 278: 1309-12.
3. Kuno Y, Abe A, Emi N, Iida M, Yokozawa T, Towatari M, et al. Constitutive kinase activation of the TEL-Syk fusion gene in myelodysplastic syndrome with t(9;12)(q22;p12). Blood 2001;97:1050-5.
4. Cazzaniga G, Tosi S, Aloisi A, Giudici G, Daniotti M, Pioltelli P, et al. The tyrosine kinase Abl-related gene ARG is fused to ETV6 in an AML-M4Eo patient with a t(1;12)(q25;p13): molecular cloning of both reciprocal transcripts. Blood 1999;94:4370-3.
5. Macdonald D, Aguiar RCT, Mason PJ, Goldman JM, Cross NCP. A new myeloproliferative disorder associated with chromosomal translocations involving 8p11: a review. Leukemia 1995; 9:1628-30.
6. Macdonald D, Reiter A, Cross NCP. The 8p11 myeloproliferative syndrome: a distinct clinical entity caused by constitutive activation of FGFR1. Bain BJ, ed. Chronic myeloproliferative disorders. Basel, Karger, 2003. p. 62-8.
7. Guash G, Popovici C, Mugneret F, Chaffanet M, Pontarotti P, Birnbaum D, et al. Endogenous retroviral sequence is fused to FGFR1 kinase in the 8p12 stem-cell myeloproliferative disorder with t(8;19)(p12;q13.3). Blood 2003; 101:286-8.
8. Grand EK, Grand FH, Chase AJ, Ross FM, Corcoran MM, Oscier DG, et al. Identification of a novel gene, FGF1OP2, fused to FGFR1 in 8p11 myeloproliferative syndrome. Genes Chromosomes Cancer 2004;40:78-83.
9. Belloni E, Trubia M, Gasparini P, Micucci C, Tapinassi C, Confalonieri S, et al. 8p11 myeloproliferative syndrome with a novel t(7;8) translocation leading to fusion of the FGFR1 and TIF1 genes. Genes Chromosomes Cancer 2005; 42:320-5.
10. Demiroglu A, Steer EJ, Heath C, Taylor K, Bentley M, Allen SL, et al. The t(8;22) in chronic myeloid leukemia fuses BCR to FGFR1: transforming activity and specific inhibition of FGFR1 fusion proteins. Blood 2001; 98: 3778-83.
11. Bain BJ, Pierre R, Imbert M, Vardiman JW, Brunning RD, Flandrin G. Chronic eosinophilic leukemia and the hypereosinophilic syndrome. In: Jaffe ES, Harris NL, Vardiman JW, eds. WHO classification of tumors: pathology and genetics. Tumors of haematopoietic and lymphoid tissues. Lyon: IARC press; 2001. p. 29-31.
12. Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med 2003;348:1201-14.
13. Vandenberghe P, Wlodarska I, Michaux L, Zachée P, Boogaerts M, Vanstraelen D, et al. Clinical and molecular features of FIP1L1-PDGFRA (+) chronic eosinophilic leukemias. Leukemia 2004;18:734-42.
14. Baxter EJ, Hochhaus A, Bolufer P, Fernandez JM, Senent L, Cervera J, et al. The t(4;22)(q12;q11) in atypical chronic myeloid leukemia fuses BCR to PDGFRA. Hum Mol Genet 2002; 11: 1391-7.
15. Mitelman F, ISCN. An International System for Human Cytogenetic Nomenclature. Basel: Karger. 1995.
16. Crescenzi B, La Starza R, Romoli S, Beacci D, Matteucci C, Barba G, et al. Submicroscopic deletions in 5q- associated malignancies. Haematologica 2004;89:281-5.
17. Baxter EJ, Kulkarni S, Vizmanos JL, Jaju R, Martinelli G, Testoni N, et al. Novel translocations that disrupt the platelet-derived growth factor receptor β (PDGFRβ) gene in BCR-ABL-negative chronic myeloproliferative disorders. Br J Haematol 2003;120:251-6.
18. Rosati R, La Starza R, Veronese A, Aventin A, Schwienbacher C, Vallespi T, et al. NUP98 is fused to the NSD3 gene in acute myeloid leukemia associated with t(8;11)(p11.2;p15). Blood 2002;99:3857-60.
19. Sohal J, Chase A, Mould S, Corcoran M, Oscier D, Iqbal S, et al. Identification of four new translocations involving FGFR1 in myeloid disorders. Genes Chromosomes Cancer 2001;32: 155-63.
20. Van Limbergen H, Beverloo B, van Drunen E, Janssens A, Hählen K, Poppe B, et al. Molecular cytogenetic and clinical findings in ETV6/ABL1-positive leukemia. Genes Chromosomes Cancer 2001;30:274-82.
21. Baens M, Peeters P, Guo C, Aerssens J, Marynen P. Genomic organization of TEL:the human ETS-variant gene 6. Genome Res 1996;6:404-13.
22. Rotoli B, Catalano L, Galderisi M, Luciano L, Pollio G, Guerriero A, et al. Rapid reversion of Loeffler's endocarditis by imatinib in early stage clonal hypereosinophilic syndrome. Leuk Lymphoma 2004;45:2503-7.
23. Frickhofen N, Märker-Hermann E, Reiter A, Walz C, Jung B, Bauer H, et al. Complete molecular remission of chronic eosinophilic leukemia complicated by CNS disease after targeted therapy with imatinib. Ann Hematol 2004;83:477-80.
24. Malagola M, Martinelli G, Rondoni M, Ottaviani E, Piccaluga PP, Ricci P, et al. Soft tissue and skeletal involvement in FIP1L1-PDGFRA positive chronic eosinophilic leukemia: imatinib mesylate may induce complete molecular and imaging remission. Haematologica 2004;89:ECR 25.
25. Pardanani A, Brockman SR, Paternoster SF, Flynn HC, Ketterling RP, Lasho L, et al. FIP1L1-PDGFRA fusion: prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe eosinophilia. Blood 2004;104:3038-45.