Systemic mastocytosis with associated myeloproliferative disease and precursor B lymphoblastic leukaemia with t(13;13)(q12;q22) involving *FLT3*

A Tzankov,[1] K Sotlar,[2] D Muhlematter,[3] A Theocharides,[4] P Went,[1] M Jotterand,[3] H-P Horny,[5] S Dirnhofer[1]

[1] Institute of Pathology, University of Basel, Switzerland; [2] Institute of Pathology, University of München, Germany; [3] Institute of Medical Genetics, University of Lausanne, Switzerland; [4] Department of Hematology, University of Basel, Switzerland; [5] Institute of Pathology, Ansbach, Germany

Correspondence to: Dr A Tzankov, University Hospital Basel, Institute of Pathology, Schoenbeinstr. 40, CH-4031 Basel, Switzerland; atzankov@uhbs.ch

Accepted 22 May 2008

ABSTRACT

Systemic mastocytoses represent neoplastic proliferations of mast cells. In about 20% of cases systemic mastocytoses are accompanied by clonal haematopoietic non-mast cell-lineage disorders, most commonly myeloid neoplasms. A case of systemic mastocytosis carrying the characteristic mutation at codon 816 (D816V) in the *KIT* gene of mast cells, with two concurrent accompanying clonal haematopoietic non-mast cell-lineage disorders, chronic myeloproliferative disease, unclassifiable and precursor B lymphoblastic leukaemia is documented. Both accompanying clonal haematopoietic non-mast cell-lineage disorders carried the wild-type *KIT* gene, but had a novel t(13;13)(q12;q22) involving the *FLT3* locus at 13q12. The chronic myeloproliferative disease, unclassifiable and the precursor B lymphoblastic leukaemia were cured by syngenous stem cell transplantation, but the systemic mastocytosis persisted for more than 10 years. The additional impact of molecular techniques on the correct diagnosis in haematological malignancies is highlighted, and evidence is provided that, apart from internal tandem duplications and mutations, *FLT3* can be activated by translocations.

Associated clonal haematopoietic non-mast cell-lineage disorders (AHNMDs) can be observed in about 20% of patients with systemic mastocytosis (SM).[1 2] SM and AHNMDs can be either clonally related or not.[3 4] Concurrent SM and precursor lymphoblastic leukaemia (ALL) have not been reported. Here, we describe a case of SM with two AHNMDs, namely chronic myeloproliferative disease, unclassifiable (CMPD, U) and B-ALL, both AHNMDs carrying a novel t(13;13)(q12;q22) involving the *FLT3* locus.

MATERIALS AND METHODS

Histology

Bone marrow biopsy samples prior to 2000 were fixed and decalcified in Susa's medium, and after 2000 in 4% buffered formaldehyde solution and EDTA; they were then embedded in paraffin. Sections (3 μm) were stained with H&E, Giemsa and Gömöri stains.

Immunohistochemistry

Immunohistochemistry for CD3, CD10, CD20, CD25, CD34, CD79a, CD117 and tryptase was performed using an automated immunostainer (Nexes, Ventana, USA), and for terminal deoxynucleotidyl transferase (TdT) and phosphorylated STAT5 manually.

Detection of the D816V mutation in the KIT gene and the V617F mutation in the JAK2 gene

DNA from the bone marrow biopsy specimens from 1996 and 2006 was evaluated for the *KIT* mutation D816V by peptide nucleic acid-mediated PCR-clamping and melting point analysis of the products as described previously.[5] DNA from microdissected mast cells from 2006 and microdissected lymphoblasts from 1996 was amplified by nested PCR and analogously analysed for *KIT* mutations.[4] PCR for the *JAK2* mutation V617F was performed on bone marrow aspirates from 1995 and 2006 as described previously.[6]

Cytogenetic analysis and t(13;13)(q12;q22) breakpoint mapping

Cell culture and chromosome preparations were standard. Chromosomes were stained in G-bands. To map the chromosomal breakpoints in t(13;13)(q12;q22), the following bacterial artificial chromosome probes were identified in the www.genome.UCSC.edu database and obtained from BACPAC Resources (Children's Hospital, Oakland, California,USA): RP11-94A1, RP11-274P12 (bridging *ZNF198* locus), RP11-80J14, RP11-367C11 (bridging *FLT3* locus), RP11-87C7 (proximal *FLT3*), RP11-85P8 (proximal/inside *FLT3*), RP11-35M5 (distal/inside *FLT3*) and RP11-89P22 (distal *FLT3*). Direct labelling of probes with FITC or Cy3 and metaphase fluorescence in situ hybridisation (FISH) was standard. Interphase FISH with Cy3-labelled RP11-87C7 and FITC-labelled RP11-35M5 was performed on bone marrow samples from the initial diagnosis of SM-AHNMD (1996) and persistent SM (2006) to determine the *FLT3* status in mast cells in consecutive FISH and tryptase-stained sections.

RESULTS

Clinicopathological case history

In November 1995, a 46-year-old man was admitted to our hospital with antiphlogistic treatment-resistant bone pain, pruritus, raised white blood cell count (16.1×10^9/l) with increased neutrophilic granulocytes (12.24×10^9/l), eosinophilia of 0.48×10^9/l and monocytosis of 1.37×10^9/l, and splenomegaly of 17.5 cm, as assessed by

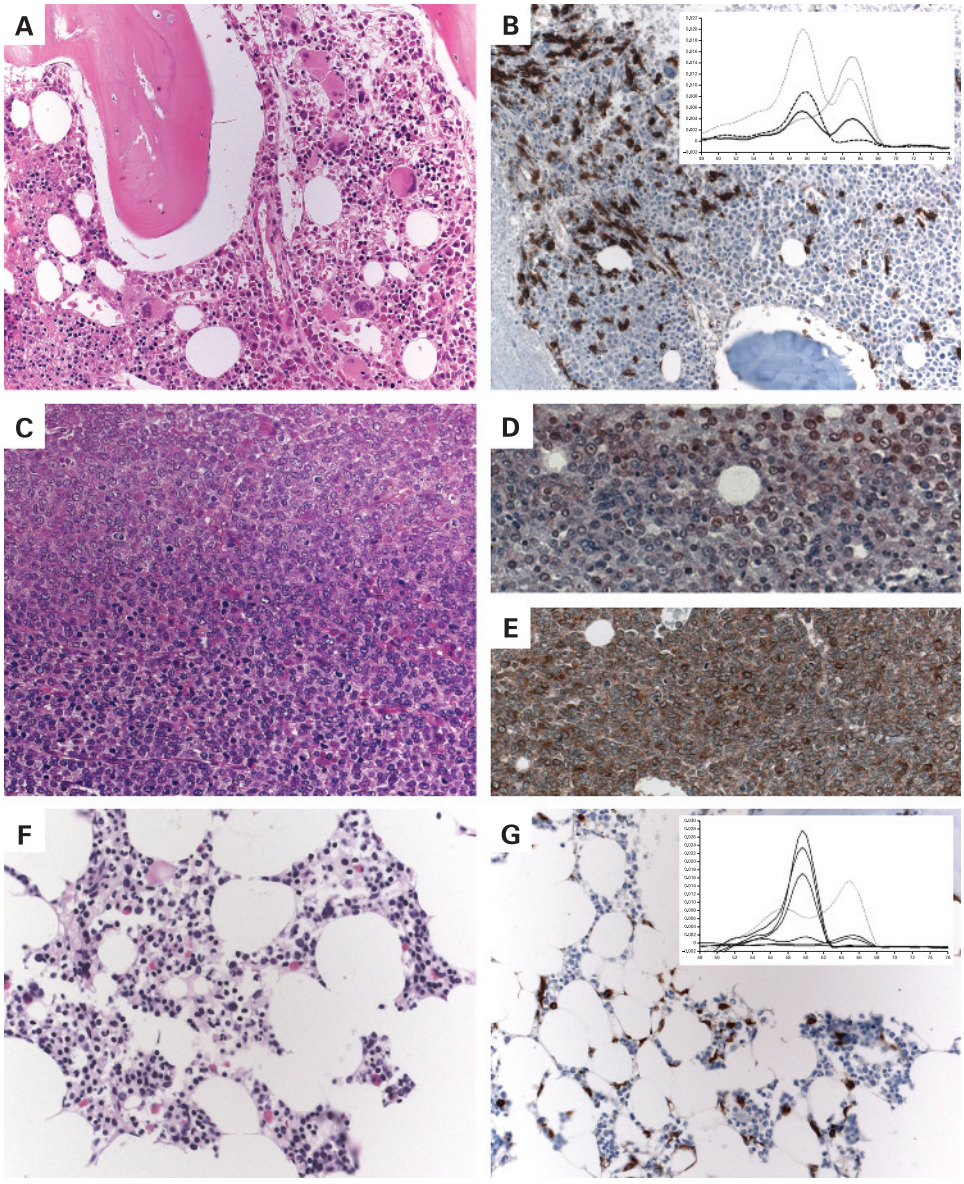

Figure 1 (A) Chronic myeloproliferative disorder, unclassified, diagnosed in 1995. Note hypercellularity, panmyelosis and atypical, partially clustering megakaryocytes; H&E, 200×. (B) CD117 staining of the same biopsy. Note grouped, partially spindle-shaped, CD117+ mast cells, diagnostic for systemic mastocytosis; immunoperoxidase, 200×. Insert: Analysis of the total DNA, extracted from the 1996 paraffin-embedded bone marrow biopsy by peptide nucleic acid (PNA)-mediated PCR-clamping and melting point analysis revealed the *KIT* mutation D816V in the initial analysis (black continuous line); black dotted line – without PNA-clamping. The mutation-specific melting peak is located at about 65°C, whereas the wild-type (wt)-specific peak is located at about 59°C. Heterozygous control DNA (grey dotted line) showed nearly complete suppression of the wt signal after PCR-clamping with a wt-specific PNA molecule (grey continuous line). (C) Precursor B lymphoblastic leukaemia (B-ALL) diagnosed in 1996. Sheets of blasts; H&E. (D) TdT- and (E) CD79-expression in B-ALL; immunoperoxidase, 400×. (F) Slightly hypocellular routine 10-year post-transplant follow-up biopsy from 2006; H&E, 200×. (G) CD117 staining of the same biopsy revealing increased amounts of mast cells; immunoperoxidase, 200×. Insert: Amplification of total DNA from microdissected mast cells from the 2006 bone marrow biopsy by PNA-mediated PCR-clamping and melting point analysis showed weak mutation-specific signals (black continuous lines; two small peaks at 65°C).

ultrasonography. The leucocyte alkaline phosphatase index was slightly decreased (15; normal range 20–100). Bone marrow aspiration was dry tap. A trephine bone marrow biopsy (fig 1A,B) showed hypercellularity of 95% with panmyelosis, megakaryocytic dysplasia and focal clustering, eosinophilia of 10%, increased perisinusoidal mast cells with clustering and a small paratrabecular population of CD20±/TdT+/CD10± precursor B-cells (<2%). Reticulin fibres were increased corresponding to myelofibrosis grade 1.[7] The morphological changes were considered consistent with CMPD, U with the possibility of accompanying SM. Due to their low numbers and lack of peripheral lymphocytosis the paratrabecular precursor B-cell aggregates did not (even retrospectively) fulfil the minimal requirements for ALL diagnosis. Conventional cytogenetic analysis of the bone marrow samples from November 1995 (and a later overt B-ALL from March 1996) revealed a t(13;13)(q12;q22) (fig 2A). Constitutional analysis (buccal mucosa) showed normal karyotype. There were no *BCR-ABL1* fusion products. Taking into consideration the clinical, imaging, laboratory and morphological findings, the patient was diagnosed with CMPD and treated with hydroxycarbamide and interferon α2a until severe leucopoenia required treatment

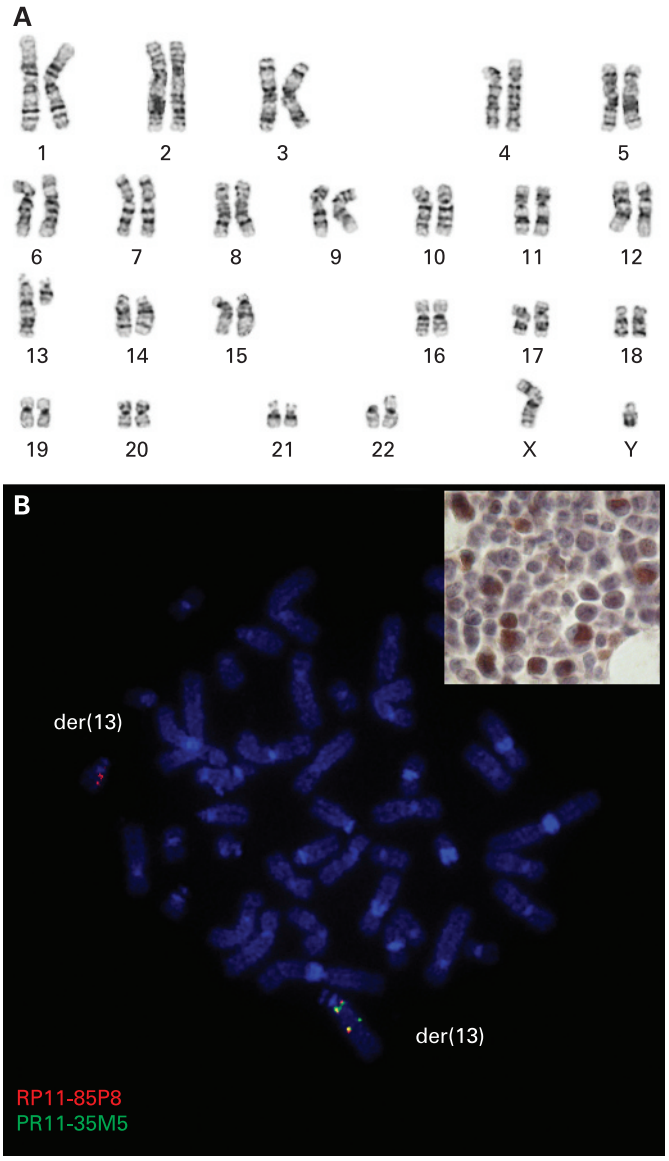

Figure 2 Cytogenetic results. (A) G-banded karyotype: 46,XY,t(13;13)(q12;q22). (B) Dual-colour metaphase fluorescence in situ hybridisation with RP11-85P8- (Cy3, red) and RP11-35M5 probes (FITC, green) showing a small red signal on the short der(13) and two fusion signals on the long der(13)(DAPI counterstaining). Insert: nuclear phosphorylated STAT5 expression in B-ALL cells from 1996 (anti-phospho-STAT5 antibody from Cell Signaling (Boston, Massachusetts, USA); dilution: 1:50, antigen retrieval: microwave, 100°C/15 min, citrate buffer pH 6).

discontinuation. Since he had a monozygous twin, syngenous bone marrow transplantation was considered.

At the admission for graft procedures atypical lymphoid cells were detected in the peripheral blood smear. Bone marrow aspiration revealed hypercellularity as well as 50% precursor B-cells with an aberrant flow cytometry-phenotype (CD19+/sIgM+/CD34+/TdT+/CD10±/CD20±/CD22+/CD43+). Trephine bone marrow biopsy revealed packed marrow with diffuse sheaths of lymphoblasts, focally displacing the haematopoiesis, as well as persistent mast cell hyperplasia and focal myeloproliferative features (fig 1C–E). The lymphoblasts were immunohistochemically CD20±/CD79a+/CD34+/TdT+/CD10±. B-ALL with reactive mast cell hyperplasia was diagnosed. The patient was treated with chemotherapy consisting of dexamethasone, vincristine, ifosphamide, carboplatin and etoposide followed by conditioning with etoposide and cyclophosphamide prior to total body irradiation and syngenous peripheral stem cell transplantation. Complete haematological remission to the last control examination in April 2008 was achieved.

In the follow-up period from 1996 to 2001, the patient complained of hyperpigmentation, relapsing pruritus, rashes, intermittent diarrhoea, and poor circulation in his fingertips. The routine 3-, 6- and 12-month post-transplant biopsies showed complete remission of B-ALL and CMPD, U and persistent minor mast cell hyperplasia. A routine 10-year follow-up trephine bone marrow biopsy in August 2006 was normocellular, with persistent complete morphological remission of B-ALL and CMPD, U. An increased amount of perivascular, focally-grouped mast cells was detected (fig 1F,G). These mast cells were tryptase+/CD117+/CD25+, indicating a neoplastic phenotype.

Genetic analysis

Dual-colour metaphase FISH carried out in 2007 on archived bone marrow cells from 1996 pointed to the involvement of the *FLT3* gene in the t(13;13)(q12;q22) (fig 2B; localised in the proximal gene part). Only the bone marrow probe from 2006 was evaluable for interphase FISH. Breaks involving *FLT3* could not be detected in 50 identifiable mast cells or 200 additional haemopoietic cells.

To study the functional consequences of the *FLT3* rearrangement, the phosphorylation status of its downstream target, STAT5,[8] was immunohistochemically analysed and compared to other acute leukaemia cases without *FLT3* abnormalities (data not shown). Nuclear phosphorylated STAT5 was detectable in 20% of B-ALL cells of the present case (fig 2B, insert), but not in blasts of acute leukaemias without *FLT3* abnormalities.

DNA analysis showed the *KIT* mutation D816V in the initial bone marrow biopsy from 1996 only with peptide nucleic acid-mediated PCR-clamping (fig 1A, insert) and in microdissected mast cells from the bone marrow biopsy in 2006 (fig 1G, insert). In contrast, the mutation was not detected in microdissected lymphoblasts from the bone marrow biopsy in 1996. *JAK2* was wild-type.

Final integrative interpretation considering molecular analyses

Taking into consideration the presence of three minor criteria (>25% spindle-shaped mast cells, co-expression of CD117 and CD25, and *KIT* mutation D816V, but normal serum tryptase, assessed only in 2006)[1] and all other molecular findings, the integrative diagnosis of persistent indolent SM with complete remission of the coexistent AHNMDs (CMPD, U and B-ALL) was finally established in 2006.

DISCUSSION

SMs in SM-AHNMDs are most commonly accompanied by myeloid malignancies and rarely by lymphoproliferative disorders, but thus far association with ALL has not been documented.[2] Importantly, clonal relationship between SM and AHNMD is currently not required to classify a coincidental haematological malignancy as AHNMD; if SM diagnosis is established, any accompanying haematological malignancy should be classified as AHNMD.[1] The observed co-occurrence of SM and ALL in our patient may be related to a possible lymphoblastic transformation (blast phase) of the initial CMPD, U. This assumption is supported by the clinical

chronology as well as by the detection of the t(13;13)(q12;q22) in both aspirates from 1995 (CMPD, U) and 1996 (B-ALL). Importantly, mast cells of SM did not carry t(13;13)(q12;q22), whereas B-ALL and CMPD, U did not carry the *KIT* mutation D816V, which is highly suggestive of differential clonal origins of SM and AHNMDs.

Regarding the novel t(13;13)(q12;q22), our observations imply that *FLT3* can be activated not only by internal tandem duplications and mutations[8] but also by translocations as in the case of another tyrosin kinase-encoding oncogene *JAK2*.[9] Although investigated on a single case, this assumption is further supported by the higher amount of phosphorylated STAT5 (flt3 downstream target)[8] in B-ALL cells in the index (fig 2B, insert) compared to reference cases (data not shown). In line with this hypothesis, a CMPD case with hypereosinophilia and t(12;13)(p13;q12) leading to *FLT3/ETV6* fusion has been reported,[10] thereby clearly demonstrating the oncogenic potential of dimerisable chimeric flt3.

Take-home messages

- Systemic mastocytosis can be accompanied by precursor cell lymphoblastic leukaemia.
- In addition to internal tandem duplications and mutations, *FLT3* can be activated by translocations.
- Systemic mastocytosis might be resistant to stem transplantation and persist after eradication of associated clonal haematopoietic non-mast cell-lineage disorders.

Competing interests: None.

REFERENCES

1. **Valent P,** Horny HP, Li CY, *et al*. Mastocytosis. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Pathology and genetics of tumours of haematopoietic and lymphoid tissues*. Lyon: IARC Press, 2001:293–302.
2. **Horny HP,** Sotlar K, Sperr WR, *et al*. Systemic mastocytosis with associated clonal haematological non-mast cell lineage diseases: a histopathological challenge. *J Clin Pathol* 2004;**57**:604–8.
3. **Sperr WR,** Walchshofer S, Horny HP, *et al*. Systemic mastocytosis associated with acute myeloid leukaemia: report of two cases and detection of the c-kit mutation Asp-816 to Val. *Br J Haematol* 1998;**103**:740–9.
4. **Sotlar K,** Fridrich C, Mall A, *et al*. Detection of c-kit point mutation Asp-816–> Val in microdissected pooled single mast cells and leukemic cells in a patient with systemic mastocytosis and concomitant chronic myelomonocytic leukemia. *Leuk Res* 2002;**26**:979–84.
5. **Sotlar K,** Escribano L, Landt O, *et al*. One-step detection of c-kit point mutations using peptide nucleic acid-mediated polymerase chain reaction clamping and hybridization probes. *Am J Pathol* 2003;**162**:737–46.
6. **Kralovics R,** Teo SS, Li S, *et al*. Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. *Blood* 2006;**108**:1377–80.
7. **Thiele J,** Kvasnicka HM, Facchetti F, *et al*. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica* 2005;**90**:1128–32.
8. **Choudhary C,** Müller-Tidow C, Berdel WE, *et al*. Signal transduction of oncogenic Flt3. *Int J Hematol* 2005;**82**:93–9.
9. **Tzankov A,** Heiss S. Myeloproliferative diseases with the recurrent t(8;9)(p23;p24) – reply. *Hum Pathol* 2006;**37**:500–2.
10. **Vu HA,** Xinh PT, Masuda M, *et al*. FLT3 is fused to ETV6 in a myeloproliferative disorder with hypereosinophilia and a t(12;13)(p13;q12) translocation. *Leukemia* 2006;**20**:1414–21.

Systemic mastocytosis with associated myeloproliferative disease and precursor B lymphoblastic leukaemia with t(13;13)(q12;q22) involving FLT3

A Tzankov, K Sotlar, D Muhlematter, et al.

J Clin Pathol 2008 61: 958-961
doi: 10.1136/jcp.2008.058073