KIT^{D816V+} systemic mastocytosis associated with KIT^{D816V+} acute erythroid leukaemia: first case report with molecular evidence for same progenitor cell derivation

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ABSTRACT

A case of systemic mastocytosis associated with a clonal haematological non-mast cell lineage disease (SM-AHNMD), where the associated disease is acute erythroid leukaemia (erythroid/myeloid type), is reported. Interestingly, molecular studies showed the KIT^{D816V+} mutation not only in the mast cells, but also in the myeloid blast population and the leukaemic erythroid cells. As is the case with most erythroid leukaemias, the patient had a very aggressive clinical course and died shortly after diagnosis. It is believed that this is the first reported case of systemic mastocytosis with erythroid leukaemia where the KIT^{D816V+} mutation was detected in all three cell types. Molecular findings provide evidence for derivation of these seemingly morphologically distinct lesions from the same clonal precursor cell. From a practice standpoint, this case illustrates the importance of definitively diagnosing the associated non-mast cell lineage disease due to its prognostic implications.

A previously healthy, adult individual, in the sixth decade of life, who had no significant past medical or surgical history, presented with fatigue. The only medication was a daily 61 mg aspirin.

A complete blood count revealed pancytopenia with rare myeloblasts identified (<1%). Overt dysplasia or circulating mast cells were not identified. Bone marrow aspirate smears showed marked erythroid predominance with increased immature precursors and dysplastic features characterised by circumferential cytoplasmic vacuolisation, nuclear contour irregularities and multilobation (fig 1A,B). Myeloblasts were increased, some smaller in size, with scant cytoplasm and minimal granulation (fig 1A,B). Mast cells were quite conspicuous and exhibited atypical spindled morphology (fig 1A,B). Megakaryocytes were decreased; rare small, dysplastic, hypolobated forms were seen.

A 500-cell differential count showed 69% erythroid precursors, 18% myeloblasts and 13% other (granulocytic precursors, lymphocytes, eosinophils). The bone marrow core biopsy was hypercellular, with abnormally thickened bony trabeculae and paratrabecular compact aggregates of spindled cells (fig 1C,D). The remaining marrow showed expanded groups of blastic mononuclear cells, many with pronormoblast cytology (round nuclei with multiple, elongated nucleoli), often associated with more mature appearing red cell precursors.

Immunohistochemistry for tryptase highlighted paratrabecular mast cell aggregates with atypical spindled morphology and increased interstitial mast cells (fig 1F). The mast cells showed aberrant CD25 positivity (data not shown). CD117 showed two patterns of staining intensity: strong positivity in the mast cells, and weaker positivity in the groups of pronormoblasts and scattered myeloblasts (fig 1E). CD34 showed increased blasts (~10–15%), and haemoglobin A showed the clusters of immature normoblasts. The presence of increased and spindled mast cells forming compact dense aggregates with aberrant CD25 expression indicated systemic mastocytosis (SM). The non-mast cell component was best classified as acute erythroid leukaemia (erythroid/myeloid type), based on the World Health Organization (WHO) 2008 criteria.

Conventional cytogenetics revealed a normal karyotype. Fluorescence in-situ hybridisation for *BCR-ABL1* was negative. Although material was available for initial flow cytometry, it was insufficient for specific cell sorting for further genetic analysis. KIT^{D816V+} mutational analysis was thus performed for further support of systemic mastocytosis and to investigate a possible clonal relationship of SM and the associated erythroid leukaemia.[1]

To specifically address the question of whether KIT^{D816V} is present in neoplastic mast cells, about 100 tryptase+ (mast) cells were individually microdissected and pooled within the tip of a PCR tube, where the cell material was digested with proteinase-K overnight and then amplified by nested PCR with outer primers KIT-1F (5′- CAC AGA GAC TTG GCA GCC AG -3′) and KIT-1B (5′- CAG GAT TTA CAT TAT GAA AGT CAC GG-3′), and inner primers KIT-2F (5′- CAG CCA GAA ATA TCC TCC TTA CT -3′) and KIT-2B (5′- TTG CAG GAC TGT CAA GCA GAG -3′).[1] For genotyping, an aliquot of the nested PCR product was analysed by the technique of ''melting point analysis'' with a pair of hybridisation probes.[2] The same procedures were performed with cells microdissected from separately immunostained slides, ie, CD34+ early progenitors/blasts, and glycophorinA+ erythroid blasts. Individually performed melting point analyses of amplification products gained from the three immunostained cell types disclosed strong signals for the activating point mutation KIT^{D816V} in tryptase+ mast cells, CD34+ progenitor cells/blasts, and glycophorin A+ ery-

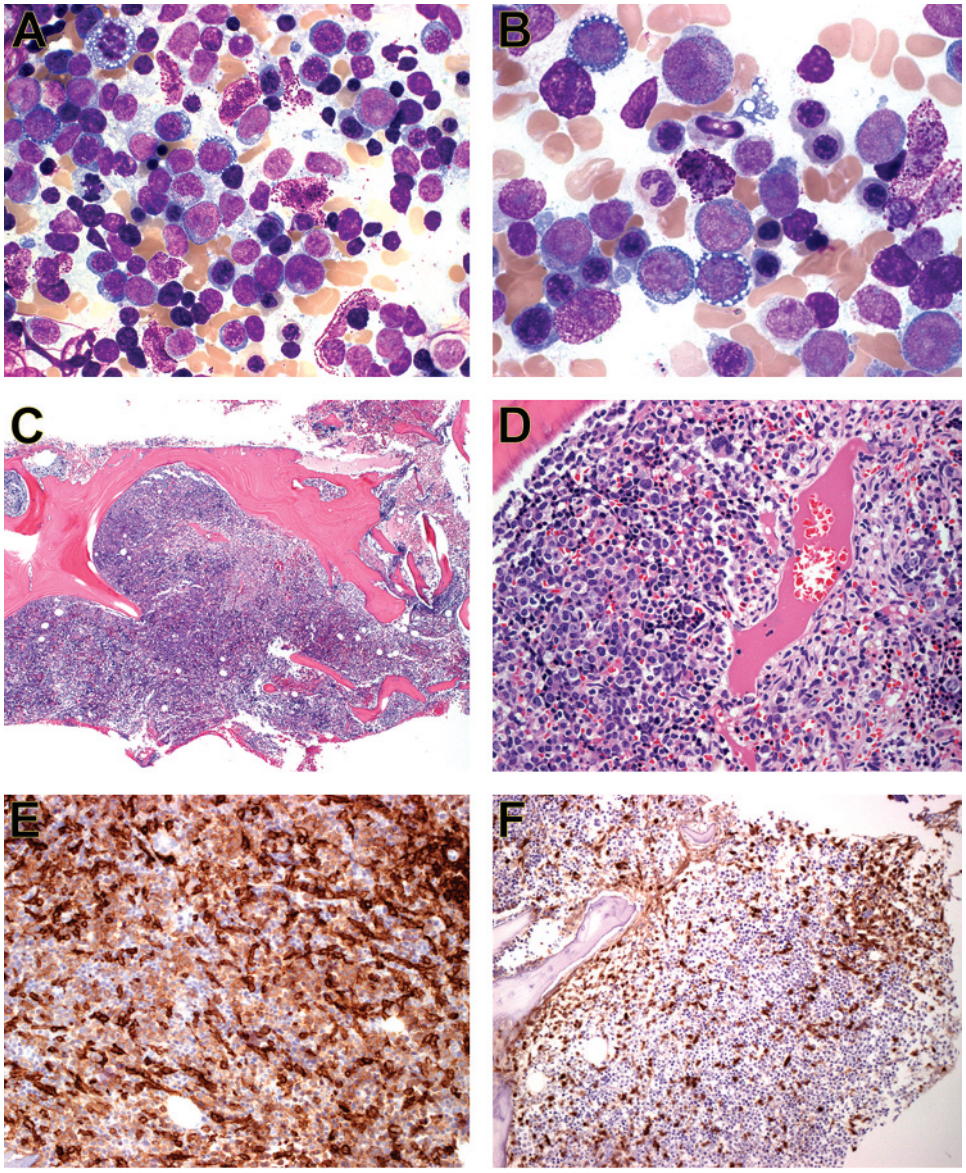

Figure 1 Bone marrow with systemic mastocytosis and associated haematological non-mast cell lineage disease. (A, B) Bone marrow aspirate showing increased and spindled mast cells, erythroid predominance with dysplasia including cytoplasmic vacuolisation, and increased myeloblasts (Wright Giemsa; original magnification ×400 and ×600, respectively). (C, D) Bone marrow core biopsy showing characteristic features of systemic mastocytosis (osteosclerosis, focal, compact aggregates of pale cells (mast cells) around bone, and associated haematological non-mast cell lineage disease (cluster of left shifted erythroids) (H&E; original magnification ×200 and ×600, respectively). (E, F) Immunohistochemical staining with CD117 and tryptase, respectively. The CD117 (E) shows both the mast cell population (dark positive) and the myeloblasts and pronormoblasts (light positive). The tryptase (F) highlights the mast cell aggregates and spindled morphology.

throblasts (fig 2A,B). For all mutational analyses, slides from formalin-fixed, paraffin-embedded bone marrow biopsy specimen were used. All experiments were repeated separately with the same results. The clinical course was very aggressive, and the patient died shortly after diagnosis.

DISCUSSION

We report the first case of systemic mastocytosis with associated clonal haematological non-mast-cell lineage disorder (SM-AHNMD) where the KIT^{D816V+} mutation was simultaneously identified in the mast cells, neoplastic erythroid cells and myeloblasts. Only rare previous studies have studied the KIT^{D816V+} mutation within the non-mast cell component. Similar to our case, Ustun *et al* utilised microdissection techniques and found the KIT^{D816V+} mutation within the neoplastic mast cells as well as the myeloid blasts representing the AHNMD.[5] These findings lend strong support to the notion that although morphologically different lesions are present; they all originally derive from the same KIT^{D816V+} progenitor clone.

In approximately 20–30% of patients with SM, AHNMD is diagnosed.[4] Both the SM and the AHNMD should be diagnosed and classified according to the World Health Organization 2008 criteria.[4] Review of the current literature shows that AHNMD primarily consists of myeloid malignancies—particularly acute myeloid leukaemia, chronic myelomonocytic leukaemia and chronic myelogenous leukaemia.[5] There are rare reports of lymphoid neoplasms, including plasma cell dyscrasia,[6] chronic lymphocytic leukaemia[7] and B-lymphoblastic leukaemia,[8] associated with SM. We now add to this list the rare occurrence of

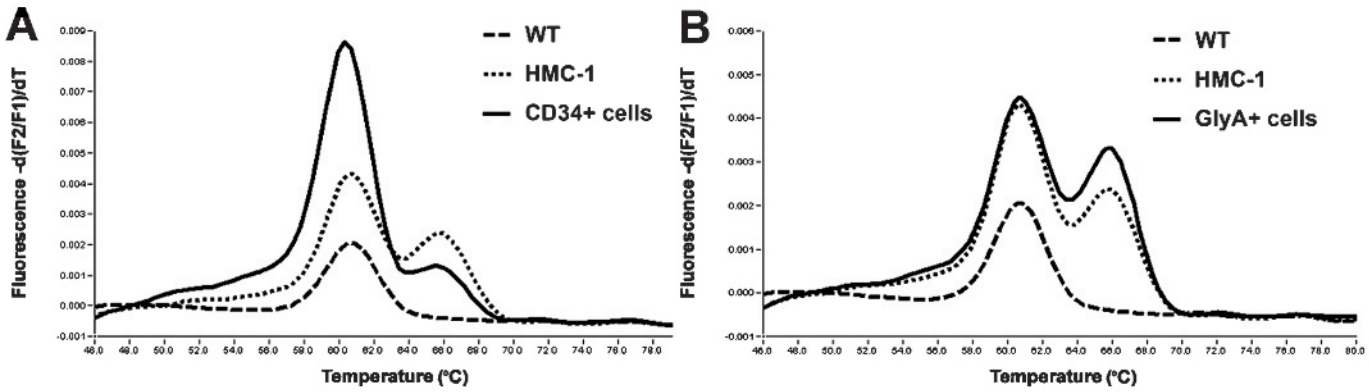

Figure 2 KIT mutation analysis. The activating point mutation D816V has a characteristic melting temperature of about 66°C. Melting point analyses of nested PCR amplified DNA from microdissected mast cells (data not shown), microdissected CD34+ progenitors/blasts (A), and microdissected glycophorin A+ cells (B) (continuous black lines) all showed a double melting peak at 60.0°C and 66°C, characteristic for the heterozygous presence of this mutation. DNA of wild-type (WT) KIT (broken line) and of a cultured mast cell leukaemia cell line (HMC-1) DNA heterozygous for the mutation D816V (dotted line) served as controls. The characteristic melting temperature of a WT PCR product is at about 60.0°C.

Take-home messages

- Although myeloid neoplasms are the usual component of systemic mastocytosis with associated haematological non-mast cell disease, a few lymphoid malignancies have also been reported.
- The KIT^{D816V+} mutation can be detected in not only the mast cells but also the associated neoplastic non-mast cell component (AHNMD).
- The non-mast cell component often has major prognostic implications.

KIT^{D816V+} acute erythroid leukaemia (erythroid/myeloid type) as the AHNMD. Overall, the prognosis of patients with systemic mastocytosis and associated acute leukaemia is quite poor as in this current case. It is has been commonly found that the KIT^{D816V+} mutation is resistant to imatinib therapy,[9] and the mast cell component often persists beyond the eradication of the non-mast cell disease. While allogeneic stem cell transplantation is considered the most effective treatment option, this may not always be practical.

We report the first case of KIT^{D816V+} erythroid leukaemia associated with SM. Biologically, our finding of the presence of the KIT^{D816V+} clone within the mast cells, neoplastic erythroids and myeloblasts supports the hypothesis of a common neoplastic progenitor. Such a progenitor has the capacity of multiphenotypic differentiation and theoretically should be eliminated in order to eradicate the disease(s). In this case, identification of the acute erythroid leukaemia component was critical as it most likely contributed to the aggressive outcome in this patient. Future studies of KIT^{D816V+} SM-AHNMD for KIT^{D816V+} AHNMD will hopefully identify additional cases derived from a single precursor clone with multilineage differentiation. Such cases will provide insight into the biological behaviour of KIT^{D816V+} disease and potentially translate into the availability of successful targeted therapies. It could be speculated that two major subtypes of SM-AHNMD exist: (1) "true" SM-AHNMD, exhibiting the activating point mutation in both the SM and the "AHNMD" compartment of the disease; and (2) SM-AHNMD, possibly those disorders associated with lymphoid neoplasms, where there is a mere coincidence between two unrelated haematological disorders.

Competing interests: None.

Patient consent: Patient data have been anonymised as patient is deceased and no relatives are listed.

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