KIT\textsuperscript{D816V}+ systemic mastocytosis associated with KIT\textsuperscript{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\textsuperscript{D816V} mutation not only in the mast cells, but also in the myeloid blast population and the leukemic 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\textsuperscript{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 paratrabeicular 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\textsuperscript{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.

To specifically address the question of whether KIT\textsuperscript{D816V} is present in neoplastic mast cells, about 100 tryptase+ (mast) cells were individually micro-dissected and pooled within the tip of a PCR tube, where the cell material was digested with protease-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 GGA GAC -3'). For genotyping, an aliquot of the nested PCR product was analysed by the technique of “melting point analysis” with a pair of hybridisation probes. The same procedures were performed with cells micro-dissected from separately immunostained slides, ie, CD34+ early progenitors/blasts, and glycoporphrin A+ 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\textsuperscript{D816V} in tryptase+ mast cells, CD34+ progenitor cells/blasts, and glycoporphrin A+ ery-
throblasts (fig 2A,B). For all mutational analyses, slides from formalin-fixed, paraffin-embedded bone marrow biopsy specimens 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. 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. Both the SM and the AHNMD should be diagnosed and classified according to the World Health Organization 2008 criteria. Review of the current literature shows that AHNMD primarily consists of myeloid malignancies—particularly acute myeloid leukaemia, chronic myelomonocytic leukaemia and chronic myelogenous leukaemia. There are rare reports of lymphoid neoplasms, including plasma cell dyscrasia, chronic lymphocytic leukaemia and B-lymphoblastic leukaemia, associated with SM. We now add to this list the rare occurrence of

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 \( \times 400 \) and \( \times 600 \), 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 erythroblasts) (H&E; original magnification \( \times 200 \) and \( \times 600 \), 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.
The KITD816V mutation associated with SM. Biologically, our finding of the presence of this mutation in this current case. It is has been commonly found that the mastocytosis and associated acute leukaemia is quite poor as in the AHNMD. Overall, the prognosis of patients with systemic mastocytosis with the non-mast cell disease. While allogeneic stem cell transplantation is considered the most effective treatment option, this 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 KITD816V 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 KITD816V mutation is resistant to imatinib therapy, 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 KITD816V erythroid leukaemia associated with SM. Biologically, our finding of the presence of the KITD816V clone within the mast cells, neoplastic erythroblasts and myeloblasts supports the hypothesis of a common neoplastic progenitor. Such a progenitor has the capacity of multiphenotypic differentiation. Such cases will provide insight into the biological behaviour of KITD816V 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.

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