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Brief communication

Chemotherapy and dasatinib induce long-term hematologic and molecular remission in systemic mastocytosis with acute myeloid leukemia with KIT^{D816V}

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ABSTRACT

Dasatinib has been reported to potently inhibit juxtamembrane domain mutant KIT^{D816V} autophosphorylation and KIT-dependent activation of down stream signaling important for cell growth and survival of neoplastic cells. Additionally, dasatinib induced apoptosis in mast cell and leukemia cell lines expressing KIT^{D816V}. Here, we present the first case report of long-term hematologic and molecular remission achieved with combined treatment with chemotherapy and dasatinib in a patient with systemic mastocytosis (SM) and acute myeloid leukemia (AML) with mutant KIT^{D816V} expression. A 50-year-old male presented with pancytopenia, organomegaly, lymphadenopathy, and lytic bone lesions in the pelvis. The patient was found to have systemic mastocytosis (SM) and acute myelogeneous leukemia (AML) positive for KIT^{D816V} and therefore diagnosed with SM with an associated clonal hematological non-mast cell lineage disease (SM-AHNMD). Both primary CD34+ cells containing myeloblasts and CD34− cells containing mastocytes obtained from the diagnostic BM lost viability markedly by *in vitro* dasatinib treatment. In addition, dasatinib diminished activity of STAT5, STAT3, AKT and ERK and attenuated the levels of c-KIT. The patient achieved a hematologic complete remission (HCR) by two induction chemotherapies with residual mastocytes. Dasatinib (70 mg PO bid, days 1–4) was added to consolidation treatments composed of four cycles of high dose cytarabine and was then continued as maintenance therapy (50 mg PO bid). Periodic bone marrow (BM) aspirate/biopsies (eight over 18 months) were performed. The patient remained in HCR, and the mastocyte burden decreased by 50%. The bone lytic lesions improved. The KIT^{D816V}mutation progressively decreased and became undetectable in the last three BM analyses. This result was confirmed by an independent laboratory showing a lack of c-KIT mutation in both CD34+ cells and CD34− cells in the last BM. No significant adverse effects of dasatinib occurred. Dasatinib has *in vitro* and *in vivo* efficacy in SM-AML patients with KIT^{D816V} mutation. Along with chemotherapy, dasatinib should be considered in these patients particularly if they cannot undergo allogeneic stem cell transplantation for this poor prognostic AML.

1. Introduction

Systemic mastocytosis (SM) is a clonal disorder of the mast cell and its precursors characterized by involvement of at least one extracutaneous organ, with or without evidence of skin infiltration. SM with an associated clonal hematological non-mast cell lineage disease (SM-AHNMD) constitutes one of the four main categories defined by the World Health Organization (WHO) [1]. The most frequently associated malignancies are myelogenous such as myeloproliferative disorders or acute myelogenous leukemia (AML) [2].

KIT, a class III receptor tyrosine kinase, consists of an extracellular domain with five immunoglobulin-like repeats, a single transmembrane domain, a juxtamembrane domain, and a cytoplasmic tyrosine kinase domain. The cytoplasmic kinase domain consists of the NH2-terminal (TK1) and COOH-terminal (TK2) lobes that are separated by a 77 amino acid hydrophilic kinase insert. The TK2 domain contains the kinase activation loop, a critical hinged

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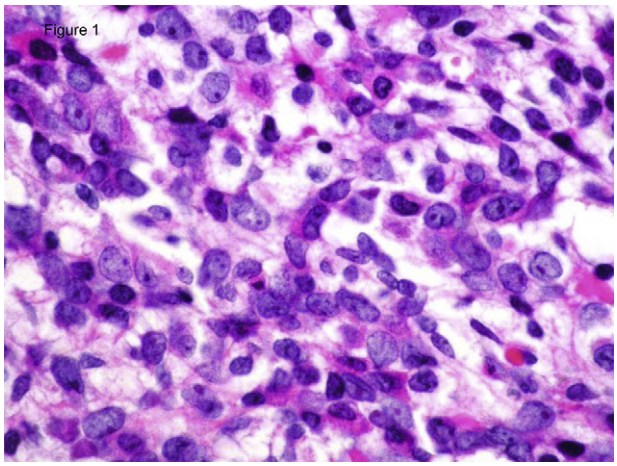

Fig. 1. The patient was diagnosed with SM and AML by a bone marrow biopsy (H&E, 100× magnification).

region of the kinase that must assume a particular conformation to allow full kinase activation [3]. The activating point mutation D816V in the KIT kinase domain is present in more than 90% of patients with SM [4].

Imatinib was found to be clinically ineffective in patients with KIT^{D816V} mutation [3,5]. In contrast, dasatinib shows *in vitro* activity against this mutant form of KIT [6–8]. *In vivo* efficacy of dasatinib in patients with SM is limited mostly to abstracts in less than 50 patients [9]. Among these patients, the number of patients with SM with AML is almost nil. All papers evaluated the response to dasatinib as a clinical response (symptoms, physical exam findings, blood count, and bone marrow morphologic change). Clinical response occurred in only approximately 30% of these patients [9]. No *in vivo* data is available on the molecular and clinical effect of dasatinib in patients with SM and AML.

We present a patient with SM and AML with the KIT^{D816V} mutation who achieved a hematologic complete remission (HCR) and complete resolution of KIT^{D816V} mutation with chemotherapy and dasatinib.

2. Methods

BM aspirate/biopsies were performed and interpreted at the Medical College of Georgia. PCR analyses of KIT^{D816V} mutation by was performed at Oregon Health and Science University. DNA was extracted within 48 h using the Qiagen mini kit (Qiagen, Valencia, CA). Bidirectional sequencing was performed on an ABI 3130 sequencer using the BigDye termination kit (Applied Biosystems, Foster City, CA).

Samples were also analyzed using a novel, quantitative, allele-specific PCR assay for KIT^{D816V}. Briefly, this assay uses a forward primer with a modified nucleotide (locked nucleic acid) that is matched at the 3′ end to the A81402T substitution (GenBank U63834). The resulting 105 bp PCR product is detected using a dual-labeled hydrolysis probe to a sequence in exon 17. As an internal control for DNA quality, the D816V PCR was multiplexed with primers and a hydrolysis probe specific for a 130 bp segment of wild-type *KIT* exon 9. Primers and probes, purchased from IDT Technologies (Coralville, IA), were as follows:

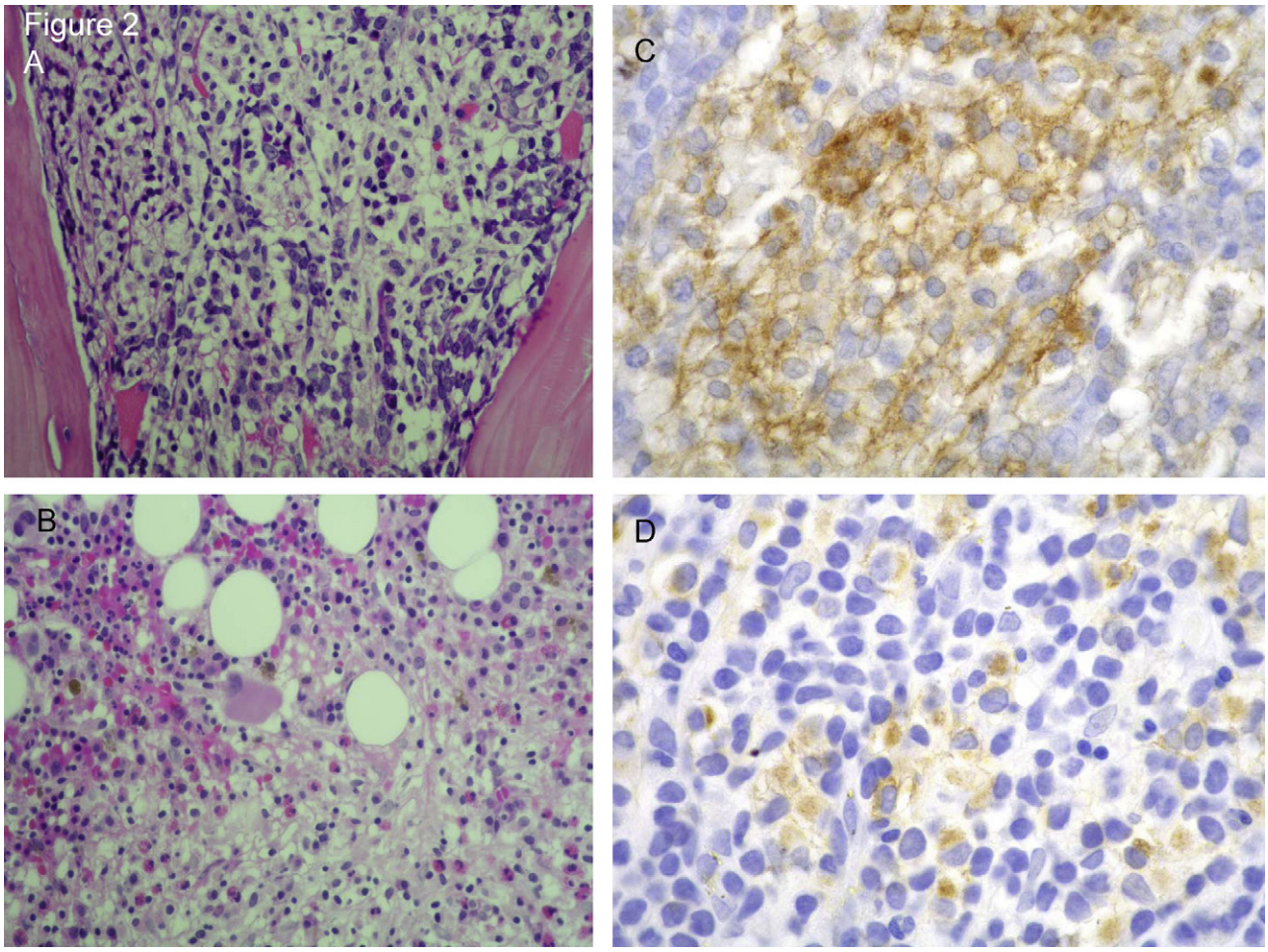

Fig. 2. Morphologically mast cells persisted but showed 50% reduction from the first to the last BM. (A) Mastocytes constituted 60% of nucleated cells. (B) Mastocytes constituted 30% of nucleated cells. (C) Compared to initial biopsy. (D) Significant decrease in CD25 positive cells after therapy.