

## Brief communication

Chemotherapy and dasatinib induce long-term hematologic and molecular remission in systemic mastocytosis with acute myeloid leukemia with KIT<sup>D816V</sup>Celalettin Ustun<sup>a,\*</sup>, Christopher L. Corless<sup>b</sup>, Natasha Savage<sup>c</sup>, Warren Fiskus<sup>d</sup>, Elizabeth Manaloor<sup>c</sup>, Michael C. Heinrich<sup>b</sup>, Grant Lewis<sup>a</sup>, Preetha Ramalingam<sup>c</sup>, Ilana Kepten<sup>b</sup>, Anand Jillella<sup>a</sup>, Kapil Bhalla<sup>a,d</sup><sup>a</sup> Medical College of Georgia, Department of Medicine, Section of Hematology/Oncology, Augusta, GA, USA<sup>b</sup> Oregon Health & Science University Cancer Institute, Portland, OR, USA<sup>c</sup> Department of Pathology, Augusta, GA, USA<sup>d</sup> Cancer Center, Augusta, GA, USA

## ARTICLE INFO

## Article history:

Received 13 June 2008

Received in revised form

22 September 2008

Accepted 24 September 2008

Available online 4 November 2008

## Keywords:

Systemic mastocytosis

Systemic mastocytosis with associated clonal hematological non-mast cell lineage disease

Acute myeloid leukemia

Asp816Val mutation

KIT<sup>D816V</sup>

Tyrosine kinase inhibitor

Dasatinib

## ABSTRACT

Dasatinib has been reported to potently inhibit juxtamembrane domain mutant KIT<sup>D816V</sup> 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<sup>D816V</sup>. 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<sup>D816V</sup> 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 myelogenous leukemia (AML) positive for KIT<sup>D816V</sup> 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<sup>D816V</sup> 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<sup>D816V</sup> 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.

© 2008 Elsevier Ltd. All rights reserved.

## 1. Introduction

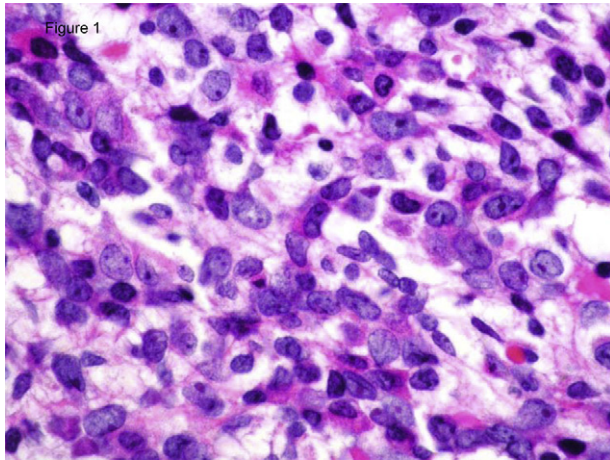
Systemic mastocytosis (SM) is a clonal disorder of the mast cell and its precursors characterized by involvement of at least one extracutaneous organ, with or without evidence of skin infiltration. SM with an associated clonal hematological non-mast cell

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 NH<sub>2</sub>-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

\* Corresponding author at: Medical College of Georgia, Department of Medicine, Section of Hematology/Oncology, 1120 15th Street, BAA-5407 Augusta, GA 30912-3125, United States. Tel.: +1 706 721 2505; fax: +1 706 721 8302.

E-mail address: [custun@mcg.edu](mailto:custun@mcg.edu) (C. Ustun).



**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

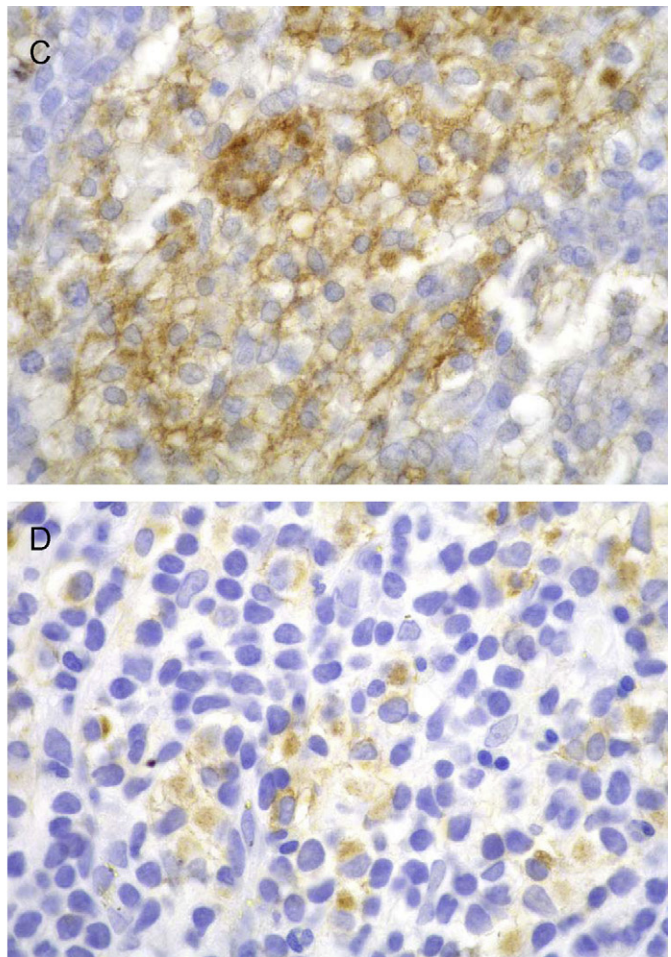
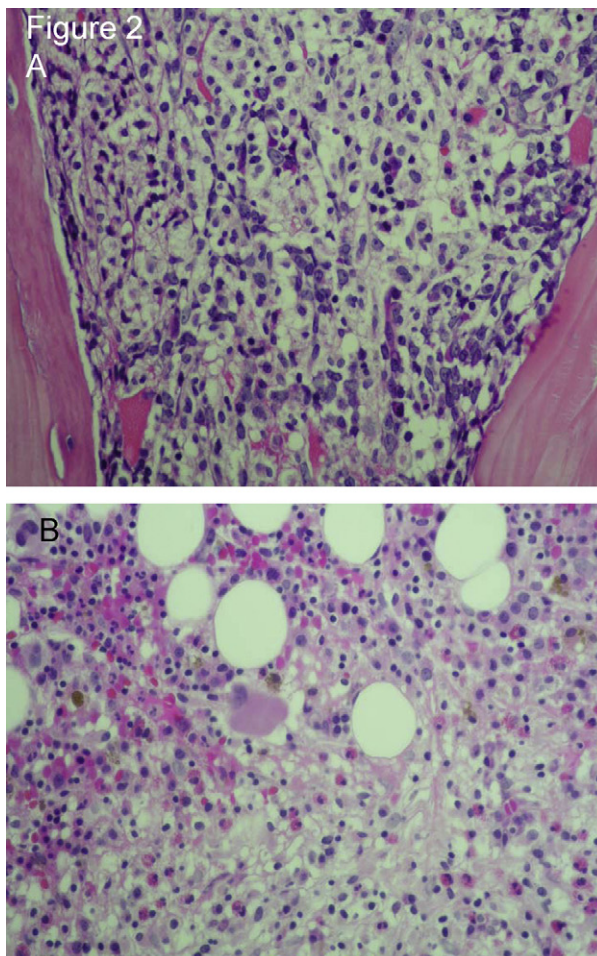
against this mutant form of KIT [6–8]. *In vivo* efficacy of dasatinib in patients with SM is limited mostly to abstracts in less than 50 patients [9]. Among these patients, the number of patients with SM with AML is almost nil. All papers evaluated the response to dasatinib as a clinical response (symptoms, 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.

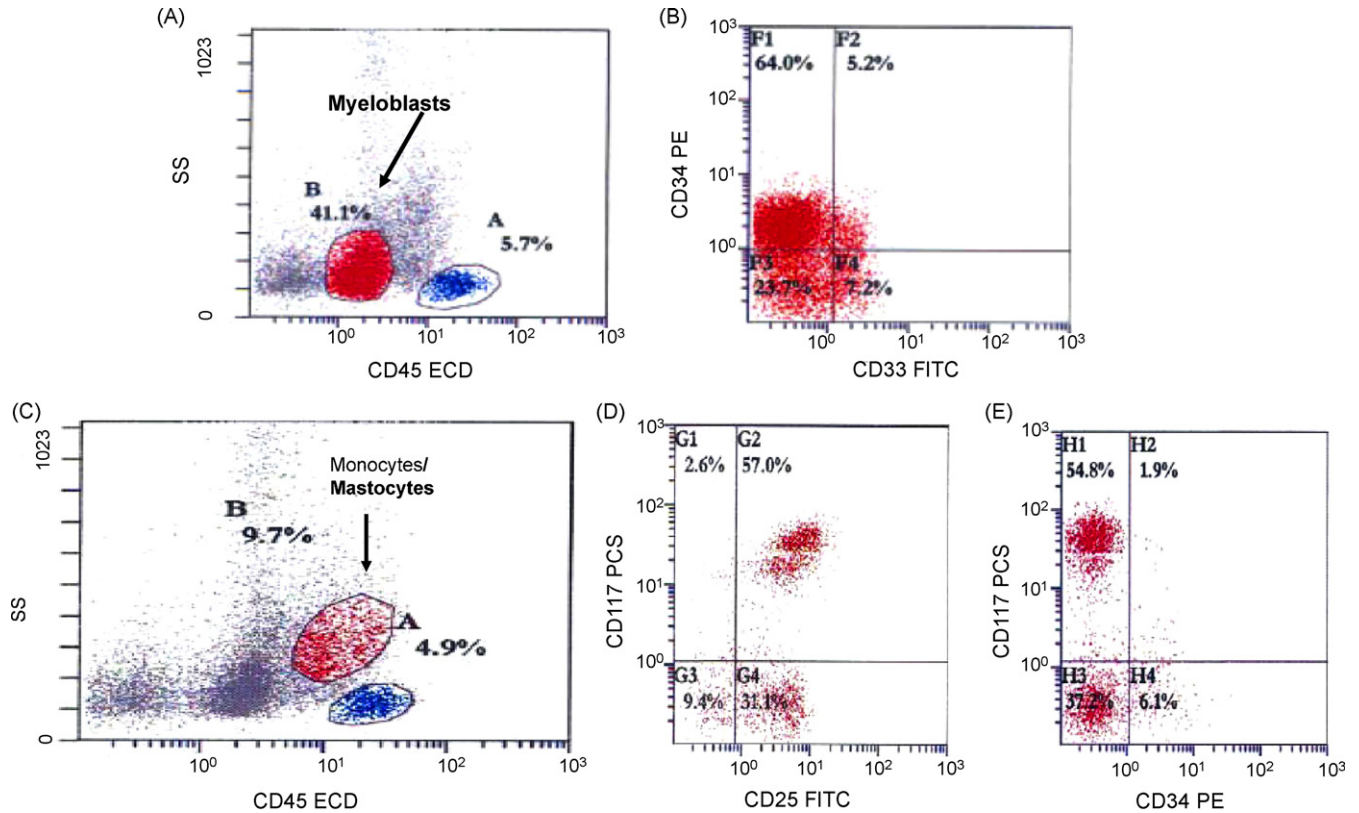
## 2. Methods

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.



**Fig. 3.** Flow cytometry (A) CD45 gating: myeloblasts were shown in red, (B) myeloblasts were positive for CD34, (C) CD45 gating: mastocytes were shown in red, (D) mastocytes were positive for CD25 and CD117, and (E) mastocytes were negative for CD34. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

KIT<sup>D816V</sup> mutation-specific (LNA) primer: GTGATTTGGCTAGCCAGAG + T (+T = LNA)  
 KIT exon 17 reverse primer: GAAACTAAAATCCITTCGAGGAC  
 KIT exon 17 dual-labeled probe: 5' FAM-TGTGGTTAAAGGAAACGTGAGTA-3' BHQ  
 KIT exon 9 forward primer: GCACAATGGCACGGTTGAAT  
 KIT exon 9 reverse primer: CAGAGCCTAAACATCCCTTA  
 KIT exon 9 dual-labeled probe: 5' TexasRed-GGCAAGACTTCTGCCTATTT-3' BHQ2

PCR was performed in 20 µl reactions on a Roche LC480 real-time system using 200 ng DNA, Roche High Fidelity polymerase and buffer, 0.45 µM primers and 0.3 µM probes. The cycling conditions were 95 °C for 10 min, then 50 cycles of 95 °C × 20 s/58 °C × 20 s/72 °C × 20 s, then 30 s of cooling at 37 °C.

To provide an estimate of the relative amount of D816V allele present, a series of standard dilutions was included in every run. DNA from the human mast cell line HMC 1.2 (heterozygous for KIT<sup>D816V</sup>) was mixed with HMC 1.1 DNA (wild-type D816) in these ratios: 1:1, 1:10, and 1:50.

**2.1. Reagents and antibodies**

Dasatinib was kindly provided by Bristol Myers Squibb (Princeton, NJ). Anti-MCL-1, anti-STAT5, anti-c-KIT and anti-STAT3 were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). Anti-pSTAT3 (Tyr 705), anti-pAKT, and anti-AKT were obtained from Cell Signaling (Beverly, MA). Anti-Bcl-x<sub>L</sub> and anti-pSTAT5 were obtained from BD Biosciences (San Jose, CA). Anti-β-actin was purchased from Sigma-Aldrich (St. Louis, MO)

**2.2. Primary AML cells**

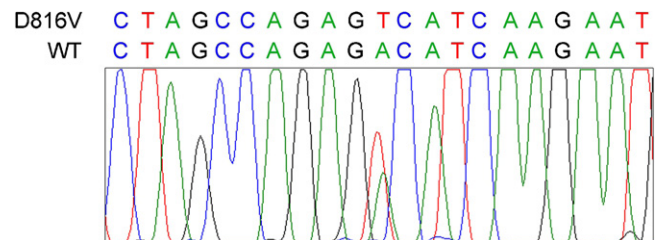
Primary acute myeloid leukemia (AML) cells were obtained with informed consent as part of a clinical protocol approved by the Institutional Review Board of the Medical College of Georgia. Bone marrow aspirate samples were collected in heparinized tubes, and mononuclear cells were separated using Lymphoprep (Axis-Shield, Oslo, Norway), washed once with complete RPMI-1640 media, re-suspended in complete RPMI-1640 and counted to determine the number of cells isolated prior to their use in the various experiments. CD34+ mononuclear cells were purified by immuno-magnetic beads conjugated with anti-CD34 antibody prior to utilization in the cell viability assay (StemCell Technologies, Vancouver, British Columbia). The CD34- cells were also reserved for cell viability assay and immunoblot analysis.

**2.3. Assessment of cell viability**

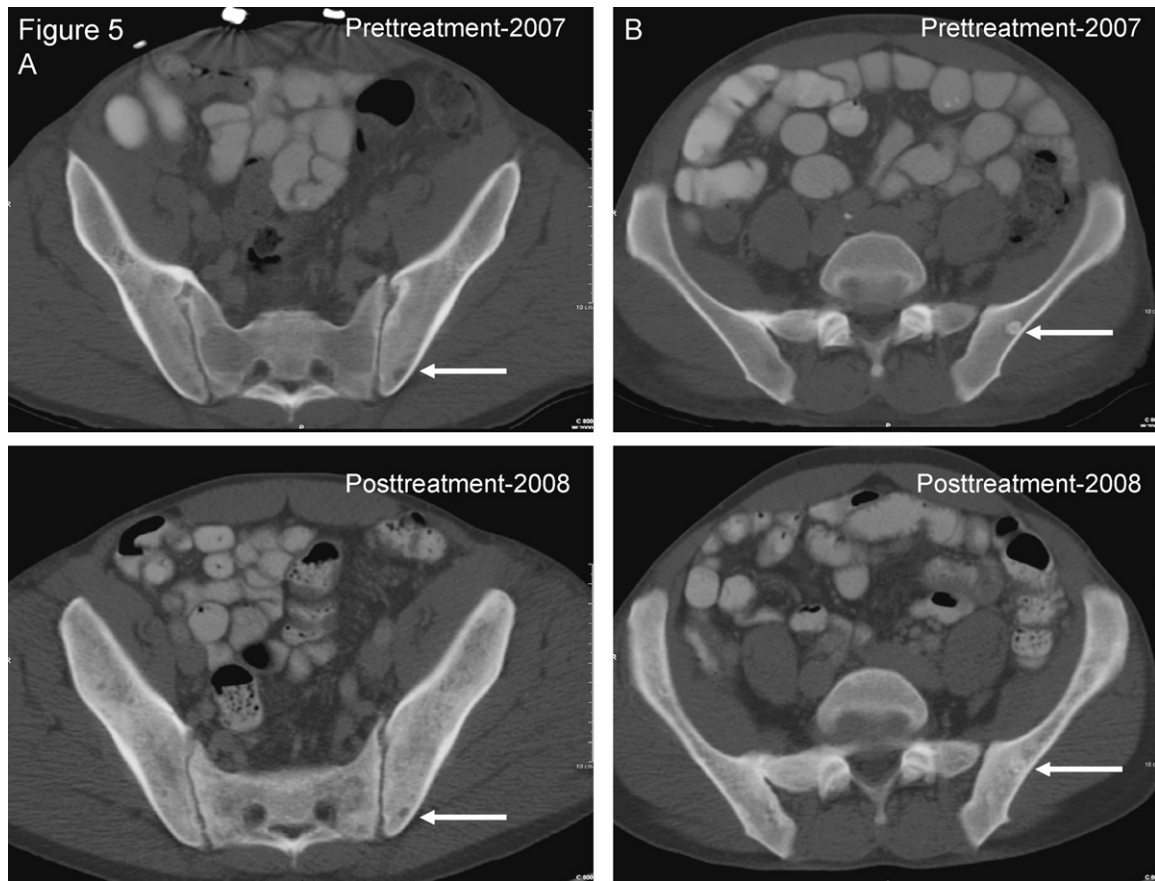
To determine the viability of primary CD34+ and CD34- cells following treatment with dasatinib, 5 × 10<sup>4</sup> CD34+ and CD34- cells were plated in 96 well plates in triplicate. Cells were treated with dasatinib for 48 h. Then 10 µL of CCK-8 solution (Dojindo, Rockville, MD) was added to each well and the plate was incubated for 4 h. Next, the absorbance for each sample was collected at 450 nm (reference filter 650 nm) and loss of viability for treated cells was compared to that of untreated control cells.

**2.4. SDS-PAGE and Western blotting**

Twenty-five micrograms of total cell lysate from untreated and treated CD34+ and CD34- cells was used for SDS-PAGE. Western blot analyses of pSTAT5, STAT5, pSTAT3, STAT3, MCL-1, pAKT, AKT, pERK1/2, ERK1/2, Bcl-x<sub>L</sub>, and c-KIT were performed on total cell lysates using specific anti-sera or monoclonal antibodies. The expression level of either β-actin was used as the loading control for the Western blots. Blots were incubated in primary antibody overnight at 4 °C. Goat anti-mouse (700 nm) and goat anti-rabbit (800 nm) secondary antibodies were incubated at 1:20,000, for 60–90 min in the dark. Blots were washed 3 × in PBST in the dark and imaged using the Odyssey Infrared Imaging System (Li-Cor Biotechnology, Lincoln, NE).



**Fig. 4.** Bidirectional sequencing of KIT exon 17 demonstrated a D816V mutation in the diagnostic BM sample.



**Fig. 5.** Lytic lesions in the pelvic bone. (A) A lytic bone lesion is smaller and shows healing. (B) A lytic bone lesion is filling in and demonstrates healing.

### 2.5. Genomic DNA isolation and quantitative determination of *c-KIT* D816V by TaqMan

Genomic DNA was extracted from CD34+ and CD34– cells using the QiaAmp DNA Blood Mini kit (Qiagen, Valencia, CA) following the manufacturer's protocol. DNA concentrations were determined using NanoDrop technology (BioLab, Auckland, New Zealand). A polymerase chain reaction (PCR) assay using TaqMan (A fluorescence-based, real-time PCR) and minor groove binding probes was designed for the detection and quantitative determination of mutant *c-KIT* (D816V) in clinical samples. Twenty nanograms of genomic DNA was added to a MicroAmp Reaction plate (Applied Biosystems, Foster City, CA) containing 20  $\mu$ L of reaction mix, custom *c-KIT* TaqMan probe, 2X Universal TaqMan master mix without AmpErase, Passive reference dye (ROX) and buffer components. The D816V mutant *c-KIT* was detected with a 5-carboxyfluorescein (FAM) fluorescently labeled probe. *c-KIT* D816V amplifications were performed using the following cycling conditions. One cycle at 95 °C for 10 min for activation of the AmpliTaq Gold, then 40 cycles of denaturation at 95 °C (15 s) and annealing/extension at 60 °C for 1 min. All samples were run in triplicate.

### 2.6. Dilution method for determining sensitivity of *c-KIT* D816V assay

DNA from an individual with AML carrying a D816V mutation was diluted with DNA from a healthy control to provide mixtures of 100, 25, 5, 1, 0.5 and 0.05% mutant *c-KIT* DNA. TaqMan *c-KIT* D816V measurement of duplicate dilutions was performed and used to determine the sensitivity of the assay to detect dilutions of the mutation.

## 3. Case report

In January 2007, a 51-year-old male presented with a 6-month history of fatigue, abdominal pain, and weight loss. An inguinal lymph node was palpated. A CBC showed WBC of  $4.5 \times 10^9/L$  with 67% blasts. A CT scan demonstrated splenomegaly and lymphadenopathy in the abdomen. A BM aspirate/biopsy revealed sheets of blasts and aggregates of round to spindle cells with abun-

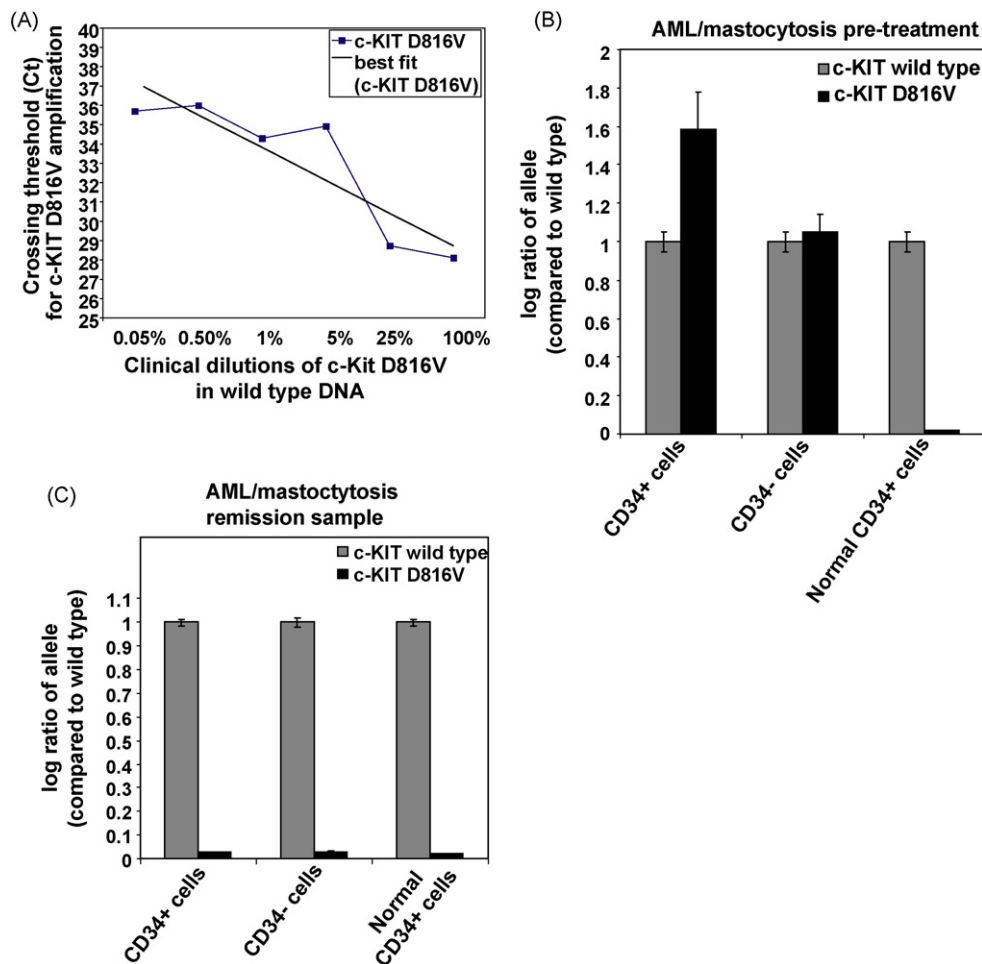
**Table 1**  
Analyses of serial bone marrow samples revealed initial diminution and ultimately complete absence of detectable D816V. Morphologically mastocytes decreased by 50% and myeloblast count decreased to <5%.

BM sample	Date	Estimated D816V allele (%)	Serum tryptase level beta/total (ng/mL)	Mast cells in BM aspirate (%)	Tryptase + mast cells in BM biopsy (%)	Myeloblasts in BM aspirate (%)	CD34+ cells in BM biopsy (%)
1	01/07	50		27	60	40	40
2	02/07	25		15	60	12	13
3	03/07	1		20	50	3	2
4	04/07	1–5		7	45	1	1
5	08/07	1		41	45	3	1
6	12/07	Undetectable		1	30	2	1
7	03/08	Undetectable	1/124	2	30	1	1
8	07/08	Undetectable	1/94	1	30	1	1

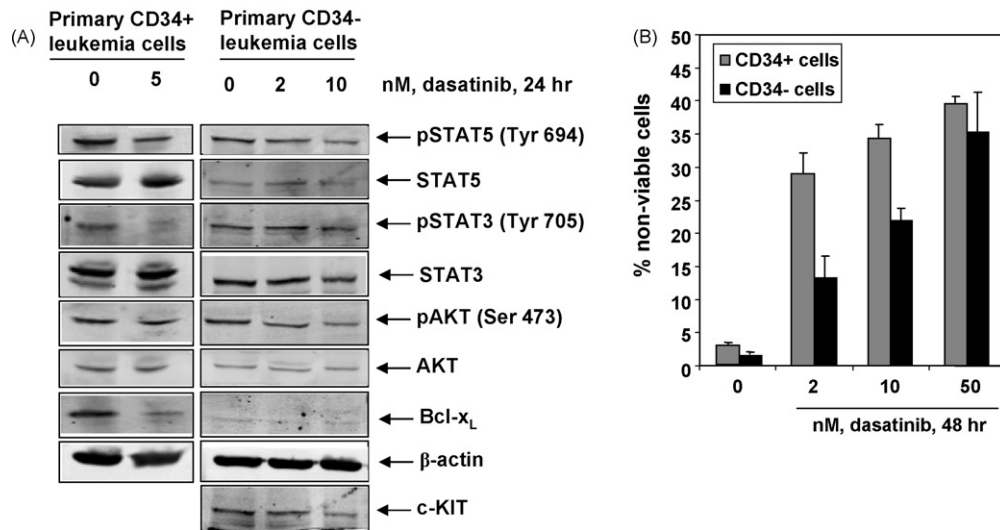
Abbreviations: BM, bone marrow.

dant clear cytoplasm consistent with mast cells (Figs. 1 and 2). Some of them were hypogranular and show elongated bean-shaped nuclei. By immunohistochemistry the mast cells were positive for CD117 and tryptase. Flow cytometry revealed two distinct populations of cells: the CD45 dim cells expressing HLA-DR, CD34, CD13, MPO and CD11c (myeloblasts) and the CD45 bright cells positive for CD64, HLA-DR, CD13, CD33, CD36, MPO, CD11c and co-expressing CD25 and CD117 (mast cells) (Fig. 3). Mast cells were negative for CD34 (Fig. 3). Cytogenetic and molecular studies revealed normal karyotype. Bidirectional sequencing of *KIT* exon 17 demonstrated a D816V mutation in the diagnostic BM sample (Fig. 4). Based on the size of the mutant peak, essentially all of the cells in the specimen contained the mutation. A CT scan of the abdomen and pelvis revealed hepatosplenomegaly, lymphadenopathy, and lytic lesions in the pelvic bone (Fig. 5). The patient was diagnosed with SM depending on one major and 3 out of 4 minor WHO criteria (e.g. >15 mast cells aggregating in the BM, spindling morphology, CD 25 positivity, and the c-KIT mutation presence). In fact, the patient has all four minor criteria (serum tryptase was not available at the diagnosis, but was found to be markedly elevated in the follow-up, Table 1). The presence of AML assigned him to

the SM-AHNMD clinicopathological category, one of the variants of SMs defined by WHO. The patient received standard induction chemotherapy with “7 + 3” for SM-AHNMD but not achieved an HCR (15% myeloblasts). The patient received re-induction with dose-attenuated high dose cytarabine (HiDAC; 2 g/m<sup>2</sup> IV every 12 h on days 1, 3, and 5) and idarubicin. The patient achieved an HCR with persistent mastocytes in the BM. Because of the following reasons dasatinib was added to the consolidation treatment and than continued as maintenance: (1) the presence of lytic bone lesions was attributed to “aggressive” SM as a “C” finding (e.g., organopathy), which required reduction therapy for mast cells, (2) The prognosis of these patients is poor [10], and allogeneic stem cell transplantation (alloSCT) is required for the treatment. AlloSCT was not available for this patient, (3) in patients with SM-AHNMD, there are evidence that AML and SM are originating from the same malignant progenitors [11,12]. Therefore this patient’s myeloblasts most likely carried the same c-KIT mutation. Dasatinib (Sprycel®, obtained from the patient support program of Bristol-Myers Squibb as a courtesy). The patient consented to off-label use of dasatinib after an extensive discussion covering potential adverse effects and benefits of dasatinib. The patient was then started on consolida-



**Fig. 6.** Sensitivity of TaqMan assay for detection of c-KIT D816V in clinical specimens of AML before and after therapeutic intervention. (A) The TaqMan assay demonstrated sensitivity for c-KIT D816V to 0.05% clinical dilution of genomic DNA mixed with normal control genomic DNA. Genomic DNA from CD34– cells was diluted as indicated and a TaqMan real-time assay was done for c-KIT D816V. The cycle at which the mutant was detected (Ct) was plotted against the dilution of c-KIT D816V DNA that was used. The black line indicates the best fit for the dilution curve. (B) CD34+ and CD34– cells were isolated from the pretreatment bone marrow of a patient with AML/mastocytosis. Genomic DNA was isolated from each cell population and 20 ng of genomic DNA was used for TaqMan assay. The graph indicates the log ratio of the D816V allele compared to the wild-type allele in each cell population. Normal CD34+ genomic DNA was used as a negative control for the D816V allele. (C) CD34+ and CD34– cells were isolated from the bone marrow of a patient with AML/mastocytosis following treatment. Genomic DNA was isolated from each cell population and 20 ng of genomic DNA was used for TaqMan assay. The graph indicates the log ratio of the D816V allele compared to the wild-type allele in each cell population. Normal CD34+ genomic DNA was used as a negative control for the D816V allele.



**Fig. 7.** Treatment with dasatinib diminishes activity of STAT5, STAT3, AKT and ERK, attenuates the levels of c-KIT and induces loss of viability in primary CD34+ and CD34– leukemia cells. (A) Primary CD34+ and CD34– cells were incubated with the indicated concentrations of dasatinib for 24 h. Then, total cell lysates were prepared and immunoblot analysis was done for pSTAT5, STAT5, pSTAT3, STAT3, pAKT, AKT, MCL-1, Bcl-x<sub>L</sub> and c-KIT. The levels of β-actin in the lysates served as the loading control. (B) Primary CD34+ and CD34– cells were plated in triplicate and incubated with the indicated concentrations of dasatinib for 48 h. Following this, cell viability was assessed using CCK-8 reagent. Columns, mean of triplicate measure; bars, SE.

tion therapy consisting of four cycles of HiDAC (3 g/m<sup>2</sup> IV every 12 h on days 1, 3, and 5) and dasatinib (70 mg PO twice a day on days 1 through 4). The patient remained on dasatinib 50 mg PO twice a day since August 2007. The follow-up BM aspirate/biopsies showed continuing HCR of AML. Morphologically mast cells persisted but showed 50% reduction from the first to the last BM (from 60 to 30%) (Fig. 2). The lytic lesions improved (Fig. 5). Follow-up biopsies were analyzed using a real-time, mutation-specific PCR assay that is sensitive to the level of 1% D816V allele. Analyses of serial marrow samples revealed initial diminution and ultimately complete absence of detectable D816V in the last three consecutive BMs (Table 1). Meanwhile the patient developed one anaphylactoid reaction (e.g. swelling in his face, lips, larynx edema) by a CT scan with no dyspnea) 5 months ago for which steroid was administered and dasatinib was held for 2 weeks.

In order to determine what cells were positive for the c-KIT mutation and if they were susceptible to dasatinib *in vitro*, his BM samples obtained from the Tumor Bank at Medical College of Georgia (the patient's BM samples were banked with his written informed consent each time). Both CD34+ cells containing myeloblasts and CD34– cells containing mastocytes were positive for the KIT<sup>D816V</sup> mutation at the diagnostic BM sample (Fig. 6). Once again, the KIT<sup>D816V</sup> mutation was found to be negative in both CD34+ and CD34– cells at the remission BM sample by a second laboratory where a more sensitive method was used (Fig. 6). Moreover, dasatinib treatment diminished activity of STAT5, STAT3, AKT and ERK and attenuated the levels of c-KIT (evaluated CD34– cells only) and induced loss of viability of CD34– cells (Fig. 7).

#### 4. Discussion

The prognosis of patients with systemic mastocytosis with AML is poor [10]. In most patients, persistence of SM even after hematologic response of the myeloid malignancy is the usual outcome [11–14]. AlloSCT appears to be effective for SM-AHNMD but was not an option for our patient [11,12]. The use of dasatinib in patients with SM is limited (<50 patients) [9,15–17]. A

Phase II study reported the result of 33 SM patients (18 indolent, 9 aggressive, and 6 with associated hematologic malignancy) who received dasatinib [9]. Nine patients improved symptomatically. Two SM-AHNMD patients (one with myelofibrosis – MF – and the other one with hypereosinophilic syndrome) achieved a CR. Neither of these two patients was c-KIT positive. The SM-MF patient later progressed to AML and died. Out of six patients, three initially showed a major response to dasatinib, but the majority (4/6) died (one due to progression to AML) [15]. A study from Europe showed symptomatic improvement in two out of four patients with SM without hematological malignancies [16]. One patient had improvement in the BM at 7 months but progressed to AML and died. A patient with SM with chronic myelomonocytic leukemia received dasatinib with symptomatic improvement [17].

Because both CD34+ and CD34– cell groups had the c-KIT mutation, in our opinion both chemotherapy and dasatinib were effective in eliminating the KIT D816V mutation in our patient. Given the fact that the patient continues to have residual mastocytes (although in reduced numbers) in the BM with no KIT<sup>D816V</sup> mutation, these results can reflect the followings possibilities: (1) Laboratory error (e.g., false negative results): very unlikely regarding the fact that the last three BM samples were consistently negative for the c-KIT mutation by a laboratory that used a PCR-based test with a sensitivity of 1%. Additionally, another independent laboratory confirmed the presence of c-KIT mutation in the diagnostic BM and the lack of the c-KIT mutation in the remission BM by using more sensitive method (0.05%), (2) chemotherapy and dasatinib combination completely eliminated the c-KIT positive myeloblasts and decreased the c-KIT positive mastocytes below the detection level of tests (e.g., greater than 3 logs) but not completely eliminated, and (3) chemotherapy and dasatinib combination completely eliminated the c-KIT positive myeloblasts and the c-KIT positive mastocytes. The remaining mastocytes do not carry the mutation. It is known that the KIT<sup>D816V</sup> mutation is not the only molecular aberration that gives a survival advantage to the neoplastic mast cells [18–20]. Dasatinib may be effective in some, but not in all aberrations [18]. Neoplastic mastocytes appear to acquire multiple genetic abnormalities, which may be responsi-

ble for its transformation to more aggressive malignancies such as SM-AHNMD (in this case AML).

The extensive and consistent laboratory data, and clinical success (e.g., long-term remission in this poor prognostic AML and improvement in the lytic bone lesions in this patient) provide convincing data which show that chemotherapy and dasatinib treatment is effective in this patient. Therefore, this combination therapy should be considered in patients with SM with AML carrying the KIT<sup>D816V</sup> mutation if they cannot undergo alloSCT.

### Conflict of interest

None.

### Acknowledgement

We would like to acknowledge Dr. Giri on behalf of the Tumor Bank team at Medical College of Georgia for providing the bone marrow samples and therefore allowing some tests to be performed latter.

**Contributions.** Celalettin Ustun contributed to idea of the study, treatment of patient, literature search, writing the paper, editing the paper. Christopher L. Corless contributed to molecular analysis, writing the paper, editing the paper. Natasha Savage contributed to extensive work in revising the paper regarding pathological aspect of it. Fiskus Warren contributed to extensive laboratory work regarding molecular tests on CD34+ and CD34– cells. Elizabeth Manaloor contributed to pathologic diagnosis and analysis of the patient, writing the paper. Michael C. Heinrich contributed to molecular analysis, literature search. Grant Lewis contributed to literature search, writing the paper. Preetha Ramalingam contributed to pathologic diagnosis and analysis of the patient, writing the paper. Ilana Kepten contributed to molecular analysis, literature search. Anand Jillella contributed to treatment of patient, writing the paper, editing the paper. Kapil Bhalla contributed to designing the extensive molecular tests, and writing and editing the paper. We also thank to Dr. Janet Munroe for her great assistance in radiologic evaluation of the case.

### References

- [1] Valent P, Akin C, Escibano L, Födinger M, Hartmann K, Brockow K, et al. Standards and standardization in mastocytosis: consensus statements on diagnostics, treatment recommendations and response criteria. *Eur J Clin Invest* 2007;37:435–53.
- [2] Sperr WR, Horny HP, Valent P. Spectrum of associated clonal hematologic non-mast cell lineage disorders occurring in patients with systemic mastocytosis. *Int Arch Allergy Immunol* 2002;127:140–2.
- [3] Foster R, Griffith R, Ferrao P, Ashman L. Molecular basis of the constitutive activity and STI571 resistance of Asp816Val mutant KIT receptor tyrosine kinase. *J Mol Graph Model* 2004;23:139–52.
- [4] Nagata H, Worobec AS, Oh CK, Chowdhury BA, Tannenbaum S, Suzuki Y, et al. Identification of a point mutation in the catalytic domain of the protooncogene c-KIT in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. *Proc Natl Acad Sci USA* 1995;92:10560–4.
- [5] Frost MJ, Ferrao PT, Hughes TP, Ashman LK. Juxtamembrane mutant V560GKit is more sensitive to Imatinib (STI571) compared with wild-type c-KIT whereas the kinase domain mutant D816VKit is resistant. *Mol Cancer Ther* 2002;1:1115–24.
- [6] Gleixner KV, Mayerhofer M, Sonneck K, Gruze A, Samorapoompichit P, Baumgartner C, et al. Synergistic growth-inhibitory effects of two tyrosine kinase inhibitors, dasatinib and PKC412, on neoplastic mast cells expressing the D816V-mutated oncogenic variant of KIT. *Haematologica* 2007;92:1451–9.
- [7] Shah NP, Lee FY, Luo R, Jiang Y, Donker M, Akin C. Dasatinib (BMS-354825) inhibits KITD816V, an imatinib-resistant activating mutation that triggers neoplastic growth in most patients with systemic mastocytosis. *Blood* 2006;108:286–91.
- [8] Schittenhelm MM, Shiraga S, Schroeder A, Corbin AS, Griffith D, Lee FY, et al. Dasatinib (BMS-354825), a dual SRC/ABL kinase inhibitor, inhibits the kinase activity of wild-type, juxtamembrane, and activation loop mutant KIT isoforms associated with human malignancies. *Cancer Res* 2006;66:473–81.
- [9] Verstovsek S, Ayalew T, Jorge C, O'Brien S, Garcia-Monero G, Pardani A, et al. Phase II Study of Dasatinib (SPRYCEL™) in Philadelphia chromosome-negative acute and chronic myeloid diseases, including systemic mastocytosis [Abstract]. *Blood* 2007;110:1039a [Abstract 3551].
- [10] Pullarkat VA, Bueso-Ramos C, Lai R, Kroft S, Wilson CS, Pullarkat ST, et al. Systemic mastocytosis with associated clonal hematological non-mast-cell lineage disease: analysis of clinicopathologic features and activating c-KIT mutations. *Am J Hematol* 2003;73:12–7.
- [11] Pullarkat V, Bedell V, Kim Y, Bhatia R, Nakamura R, Forman S, et al. Neoplastic mast cells in systemic mastocytosis associated with t(8;21) acute myeloid leukemia are derived from the leukemic clone. *Leuk Res* 2007;31:261–5.
- [12] Nagai S, Ichikawa M, Takahashi T, Sato H, Yokota H, Oshima K, et al. The origin of neoplastic mast cells in systemic mastocytosis with AML1/ETO-positive acute myeloid leukaemia. *Exp Hematol* 2007;35:1747–52.
- [13] Wong KF, Chan JK, Chan JC, Kwong YL, Ma SK, Chow TC. Concurrent acute myeloid leukemia and systemic mastocytosis. *Am J Hematol* 1991;38:243–4.
- [14] Bernd HW, Sotlar K, Lorenzen J, Osieka R, Fabry U, Valent P, et al. Acute myeloid leukaemia with t(8;21) associated with “occult” mastocytosis. Report of an unusual case and review of the literature. *J Clin Pathol* 2004;57:324–8.
- [15] Rondoni M, Paolini S, Colarossi S, Piccaluga PP, Papayannidis C, Palandiri F, et al. Response to dasatinib in patients with aggressive systemic mastocytosis with D816V Kit Mutation [Abstract]. *Blood* 2007;110:1042a [Abstract 3562].
- [16] Purtill D, Sinniah R, Cooney J, Carnley B, Cull G, Augustson B, et al. Dasatinib therapy for systemic mastocytosis: four cases. *Eur J Haem* 2008;80:456–8.
- [17] Benz R, Boesiger J, Fehr J. Systemic mastocytosis with c-KIT<sup>D816V</sup> mutation treated with dasatinib (abstract). *Blood* 2007;110:258b [Abstract 4656].
- [18] Gleixner KV, Mayerhofer M, Rix U, Hoermann G, Gruze A, Krauth MT, et al. Delineation of a KIT-independent oncogenic pathway in neoplastic mast cells that involves Lyn and Btk, and can be disrupted by the KIT/Lyn/Btk-Targeting drug (abstract). *Blood* 2007;110:460a [Abstract 1541].
- [19] Gotlib J. KIT mutations in mastocytosis and their potential as therapeutic targets. *Immunol Allergy Clin North Am* 2006;26:575–92.
- [20] Metcalfe DD. Regulation of normal and neoplastic human mast cell development in mastocytosis. *Trans Am Clin Climatol Assoc* 2005;116:185–203.