**Supplementary Information**

**Case Report**

A 42-year-old Hispanic female presented with worsening epigastric pain of five months duration, fever and 30 pound weight loss. Physical examination was notable for left upper quadrant abdominal tenderness with an enlarged spleen palpable 22 cm below the left costal margin and no palpable lymphadenopathy. An erythematous rash was present on both thighs. Hemoglobin was 7.4 g/dL, hematocrit 22.6%, platelets 111,000 /ul, and total leukocyte count 4.4 x 10^9 /l with 1% mature basophils and no blasts. Serum albumin was 3.8 g/dl (normal 3.3-5.0). CT imaging revealed a small pericardial effusion, splenomegaly and osteosclerosis throughout the spine and pelvis. Bone marrow aspirate showed both single and cohesive clusters of monocytoid cells with round to oval nuclei and a moderate amount of hypogranular cytoplasm with 62% hypogranular mast cells and no basophils (Supplementary Figure S1); biopsy revealed marked hypercellularity (100% cellularity), a diffuse monotonous infiltrate of small round to oval cells with moderate cytoplasm, minimal myeloid and erythroid elements and few megakaryocytes (Supplementary Figure S1). Flow cytometry demonstrated a myeloid population positive for CD117, CD33, dim CD45, CD13, CD2 and negative for HLA-DR, CD25, CD34 and CD38. Cytochemistry was negative for myeloperoxidase and positive for chloroacetate esterase. Karyotype was 46, XX [20]. FISH was negative for *FIP1L1-PDGFRA* fusion. *KIT* D816V mutation screened for by polymerase chain reaction (PCR) was absent. Electron microscopy demonstrated reaction product for peroxidase and “scroll-like” inclusions in the cytoplasm of some cells consistent with those seen in mast cells (Supplementary Figure S2).

Dermatologic consultation confirmed the skin finding as urticaria consistent with histamine release. Tryptase level on treatment day -4 was 514 ng/ml (normal range 0-11.4

ng/ml). Loratidine, hydroxyzine, famotidine and hydrocortisone were begun. Induction chemotherapy was begun with the CLAG regimen (cladribine 5 mg/m2/day IV over two hours on days 2-6, cytarabine [Ara-C] 2 gm/m2/day IV over four hours on days 2-6 and filgrastim 300 mcg subcutaneous daily on days 1-7) [1]. In addition, dasatinib 100 mg daily was begun on day 1. Histamine level was 218 pg/ml (range 0-1 ng/ml) on treatment day 2. On treatment day 3, she developed severe epigastric burning for one day. By treatment day 7, patient had marked improvement of splenomegaly to 11 cm below the left costal margin. On treatment day 13, she had an episode of respiratory distress and was found to have fluid overload on imaging. Bone marrow biopsy on treatment day 21 revealed persistent mast cell leukemia. Re-induction was begun with Ara-C 3 g/m2 IV over three hours daily for six days plus idarubicin 12 mg/m2 IV daily for three days. Dasatinib was discontinued on treatment day 28 secondary to gastrointestinal bleeding. On treatment day 36, CT chest revealed a moderate to severe pericardial effusion increased from admission. Persistent mast cell leukemia was again noted on bone marrow biopsy obtained on treatment day 41. Imatinib 400 mg daily was initiated for fourteen days. Bone marrow biopsy on treatment day 78 revealed persistent mast cell leukemia. The patient expired 96 days after diagnosis.

**Sample preparation, SNP array, exome capture and deep sequencing and data analysis**

Tumor genomic DNA was isolated from the initial bone marrow aspirate (74% blasts) using a Qiagen DNeasy Blood and Tissue kit. Germline genomic DNA was isolated from saliva using an Oragene saliva collection kit. Each DNA was hybridized to an Affymetrix 6.0 SNP array and data was analyzed and viewed with Nexus Copy Number version 6 software (BioDiscovery, Inc., El Segundo, CA) using the paired analysis option where the probe log-ratio value of the matching germline sample was subtracted from the tumor at each position. The B-allele frequency data was also filtered such that all probes that were homozygous in the germline

sample were first removed. After this processing, the copy number and allelic events were determined using the SNP-FASST2 algorithm.

Prior to deep sequencing, each DNA was converted into an Illumina sequencing compatible library and hybridized to probes in the NimbleGen version 2 in solution exome capture platform (SeqCap EZ Exome Library Roche NimbleGen; Madison, WI). These probes cover ~300,000 exons for a total of 44 Mb of interrogatable DNA. DNA enriched for exonic regions was sequenced on a single lane of a Genome Analyzer$_{IIX}$ instrument (Illumina) using paired-end 76 cycle chemistry.

Paired end short-read alignment was performed using the BWA (version 0.5.7-5) [2] sequence-alignment program to the human genome assembly build NCBI/hg19, with default parameters. For the germline sample, there were 37,205,349 paired end reads and for the tumor sample there were 32,799,344. After quality filtering, aligning to the genome and mapping to targeted regions, there were 24,332,956 pairs for the germline sample and 28,125,705 pairs for the tumor sample used for downstream analyses for a mean depth of 55X for the germline and 64X for the tumor. The SAMtools package [3] was used to identify SNVs in each genome. Eleven variants were validated by capillary sequencing a PCR product amplified off original tumor or germline DNA.

Thirty-eight tumor-specific SNVs were identified in the tumor genome: 5 variants outside a coding region (intron, UTR or intergenic), 12 synonymous coding changes, and 21 non-synonymous coding changes, the latter of which were further evaluated. The amino acid changes were filtered through PolyPhen-2 [4] and annotated for whether the amino acid change is predicted to be benign or damaging to the function of the protein (Table 1). Nine of the non-synonymous variants occurred in genes within the chr1p UPD region and underwent LOH. However, eight of these were not evaluated further because they either retained the reference

allele in the tumor or were present in dbSNP at high allele frequencies and are predicted by PolyPhen-2 to be benign.

This leaves 13 remaining somatic variants. One of the mutations is in the *PER3* gene which is required for CHK2 activation and apoptosis upon DNA damage [5] [6]. *KIDINS220* functions through the MEK/ERK signaling pathway and is overexpressed in melanoma [7]. *LIN7A* plays a role in establishing and maintaining the asymmetric distribution of receptors on the cell surface, including ERBB2 [8]. *LRP1B* has multiple cellular roles including regulating extracellular proteolytic activity and intracellular signaling [9] and is frequently deleted [10] or underexpressed in several tumor types [11, 12, 13, 14, 15]. *SCN4A* is a voltage-gated sodium channel that is overexpressed in ovarian cancer cells [16]. *TAF1L* plays a role in transcription [24]. The mutation in the remaining allele of *PCDH15*, Arg929Gln is in a cadherin domain [17].

Five of the mutations occurred in enzymes. *MAP3K15* (ASK3) encodes a mitogen-activated protein kinase that protects cells from undergoing apoptosis [18]. *PTPRN2* encodes a phosphatase whose promoter is hypermethylated in early stages of lung adenocarcinoma, suggesting reduction in expression levels of this protein plays a role in the transition from hyperplasia to adenocarcinoma [19]. The mutation in *PTPRN2* found here, Val876Phe, is within the tyrosine-protein phosphatase domain. *DHX9* encodes a DExH-containing ATP-dependent RNA helicase that plays multiple roles in the cell and the mutation in this case, Thr490Ala, is within one of the 8 conserved helicase signature motifs [20]. *BMP2K*, encodes a putative serine/threonine protein kinase that is induced upon BMP2 signaling in differentiating osteoblasts [21]. However, in addition to their role in bone, BMPs are cytokines that can also regulate the proliferation and differentiation of hematopoietic precursors [22] [23]. As loss of differentiation potential is a hallmark of leukemic cells, it is plausible to speculate that mutant BMP2K protein may block the differentiation of hematopoietic precursor cells.

The exome capture data generated in this study have been deposited into the NCBI Sequence Read Archive under the public accession number SRP008740.

**Primer sequences for validating SNVs available upon request.**

**Supplementary References**


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