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Letter to the Editor

Molecular response to imatinib in *KIT* F522C-mutated systemic mastocytosis

To the Editor,

Systemic mastocytosis (SM) is a neoplastic disease characterized by the multifocal accumulation and clustering of clonal mast cells in the bone marrow or other extra-cutaneous organs, a spindle-like morphology of the mast cells and an elevated serum tryptase level. Somatic mutations of the receptor tyrosine kinase encoding *KIT* gene are present in most adult SM patients with the NM_000222.2: p.(Asp816Val) mutation in exon 17, usually referred to as *KIT* D816 V, present in > 80% of cases. This mutation is an indicator of imatinib unresponsiveness [1], however, clinical responses to imatinib have been reported in SM patients with alternative, less common *KIT* mutations elsewhere in the gene [2]. While quantitation of the *KIT* D816 V mutation allele burden is valuable both for diagnosis and monitoring [3], evidence for the clinical utility of molecular monitoring in SM patients with rare *KIT* mutations is lacking.

A 69-year-old woman presented with flushing and pre-syncopal episode and was admitted to hospital with hypotension and acute renal failure. Second degree heart block was detected on her electrocardiogram and a permanent pacemaker was inserted. She re-presented shortly thereafter with further episodes of light-headedness, falls and a florid erythematous anterior chest wall rash. A full blood count revealed anaemia (Hb 9.7 g/dl) with normal vitamin B_{12} and folate levels. Given the possibility of an allergic reaction, a mast cell tryptase level was ordered and was found to be markedly elevated (247 ng/mL; normal range 2–14 ng/ml). A bone marrow biopsy demonstrated a hyper-cellular marrow with an interstitial infiltrate of oval-shaped mast cells accounting for 30% of cellularity and staining positive for CD117 (Fig. 1). Immunophenotyping detecting a population of CD117+/CD9+/CD2-/CD25- mature mast cells. The patient was commenced on H1 and H2 blockers and a mast cell-stabilising agent pending molecular investigations. The *KIT* D816 V mutation was not detected by droplet digital PCR (ddPCR) screening (PrimePCR mutation assay, Bio-Rad Laboratories, Hercules CA, USA), however, given the highly suggestive mature oval morphology and the CD2-/CD25- immunophenotype, mutational hotspots of *KIT* exons 2, 8, 9, 10, 11 and 17 were sequenced using a custom-designed next-generation sequencing assay on an Illumina MiSeq (Illumina, San Diego CA, USA). A single activating mutation was detected: NM_000222.2: c.1565 T > G; p.(Phe522Cys); hereafter referred to as *KIT* F522C [4]. Next-generation sequencing using a panel of 56 genes (Illumina TruSight myeloid panel) recurrently mutated in myeloid malignancies detected no additional mutations.

As SM with the *KIT* F522C mutation had previously been reported to be responsive to imatinib [5], the patient was commenced on this TKI at a dose of 400 mg once daily. By day 15, her serum tryptase had fallen by more than 50% with resolution of the pre-syncopal episodes and rash. A bespoke ddPCR assay with a limit of detection of approximately 0.01% mutated alleles for *KIT* F522C was designed to monitor molecular response. The ddPCR reaction mixture consisted of 10 µl ddPCR supermix (Bio-Rad, Watford, UK), 250 nm wild type probe ([HEX]-ACCC[+T]GT[+T]CA[+C]TC[+C]TTT[BHQ1]), and mutation specific probe ([6FAM]ACCC[+T]GT[+G]CA[+C]TC[+C]TTT[BHQ1]), 900 nm forward (CCACATTTCTCTTCCATTG) and reverse (AGGTCAG AATCATCACAATA) primers, and 7.2 µl of fragmented DNA at 16.25 ng/µl (bases in square brackets are locked nucleic acids). The entire reaction was loaded into a cartridge (Bio-Rad) together with 70 µl droplet generation oil (Bio-Rad) and placed into the droplet generator (Bio-Rad). After processing, the droplets were transferred to a 96 well plate which was sealed. PCR amplification was performed in a thermal cycler (Applied Biosystems 2720) using the following cycling conditions: 95 °C for 10 min., 40 cycles of 94 °C for 30 s, 58.3 °C for 30 s, 72 °C for 30 s and 1 cycle of 98 °C for 10 min. and ending at 4 °C. After amplification, the plate was loaded onto the droplet reader (Bio-Rad) and analysed immediately with Quantasoft analysis software (Bio-Rad). The mutation allele burden had fallen from 10.0% pre-treatment to 1.3% on day 15 of imatinib and to 0.03% by day 120, in parallel with normalisation of the serum tryptase level (Fig. 2). The imatinib dose was reduced to 300 mg once daily with the *KIT* F522C mutation undetectable by ddPCR at 15 months after starting imatinib. The patient is asymptomatic, remains on imatinib and has a mild anaemia which is being treated with erythropoietin.

While most patients with SM harbour a *KIT* D816 V mutation, several other *KIT* mutations have been described with F522C, located in the trans-membrane domain encoding region, accounting for only 1% of all SM-associated *KIT* mutations [1]. The morphological, immunophenotypic and molecular findings support a diagnosis of aggressive, well-differentiated SM, a distinct histopathological entity, previously associated with non-D816 V *KIT* mutations and responsiveness to imatinib [5,6]. Serial monitoring of serum tryptase level is a proven method to monitor the early therapeutic response in SM patients but is of limited value once levels return to the normal range [7]. Identification of the *KIT* F522C mutation offered a means to monitor continued therapeutic efficacy with a disease-specific marker at a superior sensitivity to that of serial measurement of serum tryptase. Additional molecular aberrations are present in most patients with advanced *KIT* D816V-mutated SM though are less frequent in cases classified as indolent or smouldering SM. These additional mutations suggest a complex underlying clonal architecture with mutations in *SRSF2*, *ASXLI* or *RUNX1* being associated with an aggressive clinical course and a poor prognosis [8]. The lack of additional detectable mutations in the patient described herein suggests a molecularly naïve disease, potentially driven solely by the *KIT* F522C mutation as evidenced both by the rapid normalisation of the serum tryptase and by the molecular response to single-agent imatinib.

In this case, identification of a *KIT* mutation other than D816 V allowed selection of an appropriate tyrosine kinase inhibitor and provided an additional, sensitive approach to assess residual disease.

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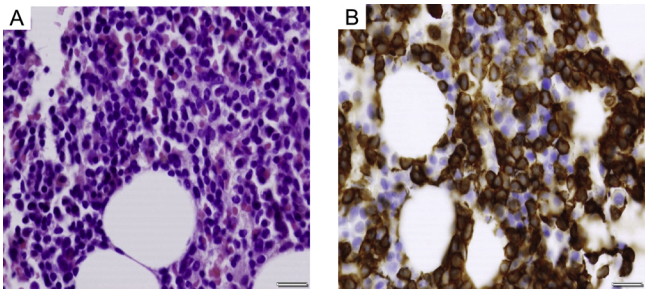

Fig. 1. Bone marrow morphology demonstrating infiltration of mature, well differentiated, oval mast cells. A: Haematoxylin and eosin; B: CD117 immunostaining.

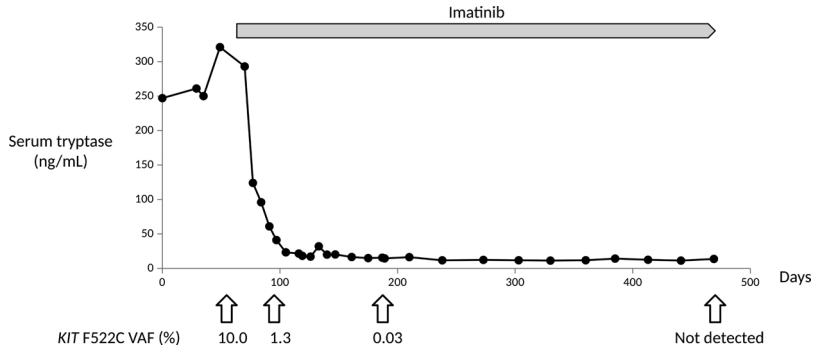

Fig. 2. Serum tryptase measurements and *KIT* F522C allele burden by digital droplet PCR (ddPCR) throughout disease course. VAF: variant allele frequency.

Consent

Written informed consent was obtained from the patient.

Conflict of interest statement

All authors declare no conflicts of interest.

Author contributions

VB and PJH provided patient care and clinical information; KW and NCPC provided and performed molecular investigations; VB and SEL collated data and drafted the manuscript; MJ performed histopathological evaluation. All authors critically reviewed the manuscript and approved the final submitted version.

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