# Increased detection of *KIT* D816V mutation in peripheral blood samples from patients with indolent systemic mastocytosis (ISM) in part 1 of the phase 2 PIONEER study using a high sensitivity droplet digital (dd) PCR assay compared with next-generation sequencing (NGS)


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## Background

- Systemic mastocytosis (SM) is a rare, clonal mast cell (MC) neoplasm driven by the *KIT* D816V mutation in ~95% of cases and characterized by severe and debilitating skin, gastrointestinal, and systemic symptoms, including potentially life-threatening anaphylaxis, which are caused by MC degranulation and mediator release[1–3]
- Diagnosis of SM by WHO classification[4] including 1 major plus 1 minor criterion or 3 minor criteria (1 being *KIT* D816V mutation[a]) can be challenging, easily missed, and delayed by years after disease onset[5]
- Next-generation sequencing (NGS) often lacks sufficient sensitivity to detect *KIT* D816V, leading to potential misdiagnosis
- A highly sensitive assay[6] such as droplet digital polymerase chain reaction (ddPCR) is essential to reliably detect the *KIT* D816V mutation allele fraction (MAF), especially in peripheral blood (PB) samples[7]

[a]In the bone marrow (BM) or another extracutaneous organ.

## Methods

- Central ddPCR (limit of detection [LOD] 0.02%) and NGS assays (LOD 1.0%)[a] were compared for the measurement of *KIT* D816V MAF in PB samples and BM aspirates (central ddPCR) or BM aspirates only (NGS) of patients with indolent SM inadequately controlled by supportive care (**Table 1**)
  - Enrolled in part 1 of the randomized, double-blind, placebo-controlled, phase 2 **PIONEER study** (**NCT03731260**)
  - Treated with avapritinib, a highly potent and selective inhibitor of KIT D816V mutant kinases (**Figure 1**)

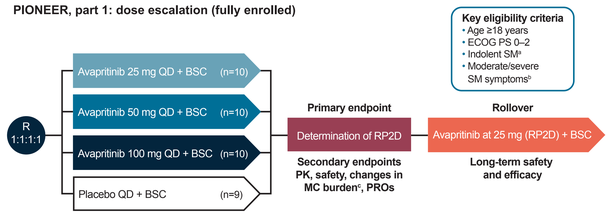
[a]Central ddPCR assay detected *KIT* D816V and central NGS (TruSight™ Myeloid Panel) detected *KIT* D816V and other co-mutations. Local quantitative and qualitive *KIT* testing was performed in PB or BM and was generally qualitative (present/absent).

### Table 1: *KIT* D816V detection assays

| KIT D816V detection assays used in this study include: | |
|---|---|
| **ddPCR**<br>• Performed using the BIO-RAD PrimePCR™ c-*KIT* D816V mutation assay in conjunction with the BIO-RAD QX200 ddPCR system<br>• Performed on PB and BM aspirate samples (MolecularMD, Portland, OR) | **NGS**<br>• Illumina TruSight® assay covers over 15 full genes (exons only) and 39 additional genes with hotspot coverage<br>• Includes genes of interest for SM: *SRSF2*, *ASXL1* and *RUNX1*<br>• Performed on BM aspirates (MolecularMD, Portland, OR) |

BM, bone marrow; ddPCR, droplet digital polymerase chain reaction; NGS, next-generation sequencing; PB, peripheral blood; SM, systemic mastocytosis.

### Figure 1: Study design




[a]Confirmed by central pathology review of bone marrow biopsy and central review of B- and C-findings, according to World Health Organization criteria. [b]Based on minimum mean TSS over the 14-day eligibility screening period despite ≥2 classes of BSC medications. [c]Measured by reduction of serum tryptase, peripheral blood *KIT* D816V allele fraction and bone marrow MC. BSC, best supportive care; MC, mast cells; PK, pharmacokinetics; PROs, patient reported outcomes; QD, once daily; R, randomization; RP2D, recommended phase 2 dose; TSS, total symptom score.

## Results

### Table 2: Patient baseline and clinical characteristics

| Patient demographics | All doses (n=39) |
|---|---|
| Median age (range), years | 51 (21–75) |
| Female, n (%) | 30 (77) |
| ECOG PS, n (%) | |
| 0 | 12 (31) |
| 1 | 19 (49) |
| 2 | 8 (21) |
| **SM therapy, n (%)** | |
| Prior cytoreductive therapy[a] | 6 (15) |
| Baseline supportive care medications, median (range) | 4 (2–9) |
| H1 blockers | 37 (95) |
| H2 blockers | 30 (77) |
| Leukotriene receptor antagonists | 23 (59) |
| Proton pump inhibitors | 18 (46) |
| Cromolyn sodium | 12 (31) |
| Corticosteroids | 6 (15) |
| Omalizumab | 9 (23) |
| **Patient disposition** | |
| Weeks on study median (range) | 18 (1–36) |
| Still on study, n (%) | 37 (95) |
| Discontinued study, n (%) | 2 (5) |
| Patient decision, n | 1 |
| Protocol non-compliance, n | 1 |
| **Mast cell burden** | |
| Central diagnosis of indolent SM, n (%) | 39 (100) |
| Median TSS score (range) | 52 (19–100) |
| Tryptase (central) ng/mL | |
| Mean (SD) | 84 (101) |
| Median (range) | 45 (6–416) |
| <11.4 ng/mL, n (%) | 3 (8) |
| 11.4 to 20 ng/mL, n (%) | 6 (15) |
| >20 ng/mL, n (%) | 30 (77) |
| Bone marrow core biopsy MC (central), % | |
| Mean (SD) | 16 (16) |
| Median (range) | 10 (1–60) |
| MC aggregates present, % | 90 |
| BM MC count[b], n (%) | |
| 1–9% | 14 (36) |
| 10–15% | 12 (31) |
| 16–30% | 8 (21) |
| 31–60% | 4 (10) |

| *KIT* D816V mutation | Local[c] | Central NGS[d] | Central ddPCR[e] |
|---|---|---|---|
| n (%) detected | 31 (80) | 11 (28) | 37 (95) |
| Median MAF, % (range) | – | 11 (1.9–32) | 0.36 (0.02–30.22) |

Based on data cut-off date of December 27, 2019.
[a]Included midostaurin, imatinib, dasatinib, masitinib, and interferon alfa. [b]BM MC was not detected in one patient. [c]Local quantitative and qualitative *KIT* testing of bone marrow and/or blood, various methods and sensitivities. [d]NGS targeted myeloid panel (central) in bone marrow samples, algorithmic calling sensitivity to 1.0% MAF; median MAF and range at screening. [e]ddPCR in blood (central), sensitivity to 0.02% MAF, detected: positive at screening or C1D1, median MAF and range at C1D1 in those with any detection. C1D1, cycle 1 day 1; ECOG PS, Eastern Cooperative Oncology Group performance status; SD, standard deviation.

### Table 3: Confirmed diagnostic characteristics of enrolled patients

| | Placebo (n=9) | Avapritinib 25 mg (n=10) | Avapritinib 50 mg (n=10) | Avapritinib 100 mg (n=10) | Overall (N=39) |
|---|---|---|---|---|---|
| **Major diagnostic criterion, n (%)** | | | | | |
| Dense MC aggregates in BM | 8 (89) | 10 (100) | 9 (90) | 8 (80) | 35 (90) |
| **Minor diagnostic criteria, n (%)** | | | | | |
| >25% irregular shaped MC[a] | 9 (100) | 10 (100) | 10 (100) | 8 (80) | 37 (95) |
| *KIT* D816V mutation[b] | 9 (100) | 10 (100) | 10 (100) | 8 (80) | 37 (95) |
| Serum tryptase >20 ng/ml | 7 (78) | 7 (70) | 8 (80) | 7 (70) | 29 (74) |
| CD25 expression on MC | 9 (100) | 10 (100) | 10 (100) | 9 (90) | 38 (97) |

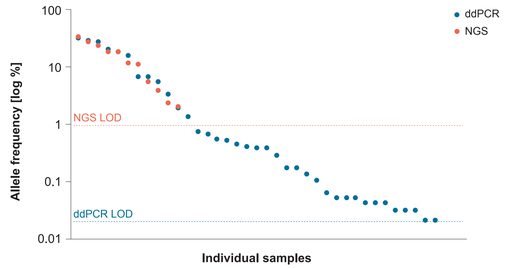
[a]The percentage of immature and atypically shaped MC were assessed. [b]Performed at baseline by ddPCR of peripheral blood.

- The high-sensitivity ddPCR assay method demonstrated:
  - *KIT* D816V mutation detection in 95% of PB samples from patients with previously confirmed ISM (**Table 4**)
  - 30-fold greater sensitivity over NGS for measuring MAF; median percentage MAF (range) was 0.36 (0.02–30.22) by ddPCR and 11 (1.9–32) by NGS (**Figure 2**)
  - Greater diagnostic sensitivity for ISM compared with serum tryptase >20 ng/mL (77%) and presence of BM MC aggregates (90%)

### Table 4: *KIT* D816V mutational status by detection assay

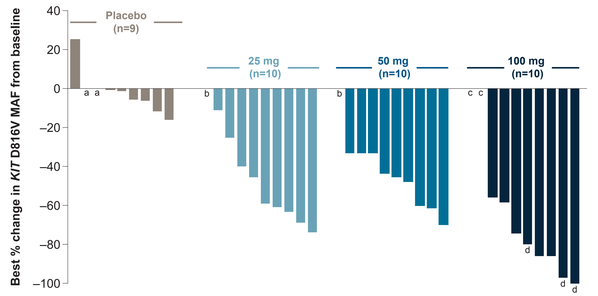
| | Local assessment n (%) | TruSight NGS n (%) | ddPCR n (%) |
|---|---|---|---|
| *KIT* D816V detected | 30 (80) | 11 (28) | 37 (95) |
| *KIT* D816V not detected | 9 (20) | 28 (72) | 2 (5) |
| Patients analyzed | 39 | 39 | 39 |

### Figure 2: Greater sensitivity for the detection of *KIT* D816V MAF was observed using ddPCR than NGS




Results were expressed as the percentage of patient PB samples testing positive for *KIT* D816V mutation (all genomic assays) and the log percent of MAF as measured by both central assay methods. NGS data at screening and ddPCR values at C1D1 were plotted for the scatter graph.

### Figure 3: Objective reductions in *KIT* D816V MAF were observed across all tested avapritinib doses




[a]No change from baseline in *KIT* D816V MAF; values above LOD. [b]Reported as no change from baseline due to MAF values being at or below LOD. [c]No detection of *KIT* D816V MAF at screening or C1D1. [d]MAF levels were reduced below LOD or not detected at best reduction.

## Conclusions

- The ddPCR PB assay method demonstrated greater sensitivity (LOD 0.02% MAF) for the detection of the *KIT* D816V mutation than BM NGS (LOD 1.0% MAF); ddPCR assay detected *KIT* D816V mutation in 95% (37/39) of patients with moderate/severe ISM
- These results highlight the clinical value of ddPCR-based measurement of *KIT* D816V mutation burden in PB as a confirmatory diagnostic tool to facilitate identification of patients with ISM
- The highly sensitive ddPCR PB assay may also be useful as a potential non-invasive, screening assay for identifying patients with suspected SM that would require a confirmatory BM biopsy
- The ddPCR assay could also be used to therapeutically monitor patients with ISM during the course of therapy with highly specific tyrosine kinase inhibitors, such as avapritinib


### References

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