# Ultra-sensitive Testing Identifies Previously Undetected *KIT* D816V Mutations in Patients With Indolent Systemic Mastocytosis: Learnings From the PIONEER Study of Avapritinib

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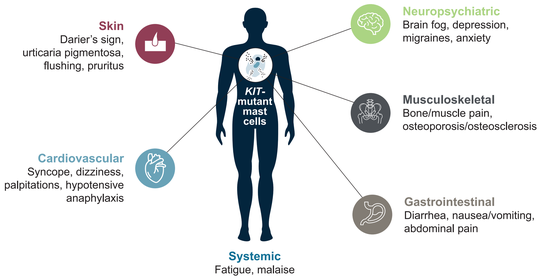
## Introduction

- Indolent systemic mastocytosis (ISM) is the most common subtype of systemic mastocytosis (SM) and is characterized by long-term, debilitating cutaneous, gastrointestinal, neurological, and musculoskeletal symptoms (**Figure 1**)[1–5]

### Figure 1. Clinical manifestations of ISM




ISM, indolent systemic mastocytosis.

- The prevalence of SM has been estimated at up to 1 in 5000 people[6–9]
- ISM is driven by the *KIT* D816V mutation in ~95% of patients and the presence of this mutation is one of the four minor diagnostic criteria for SM[10–16]
- Droplet digital polymerase chain reaction (ddPCR) is the current gold standard test for the detection of the *KIT* D816V mutation (**Table 1**)

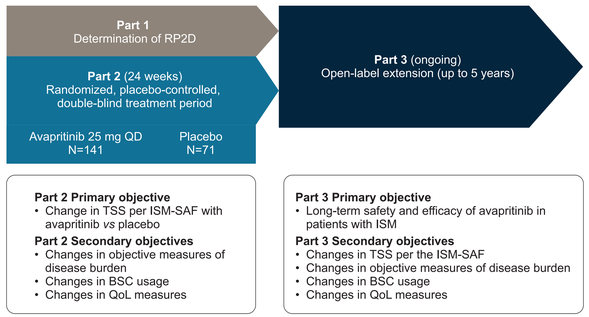
### Table 1. Testing modalities for *KIT* D816V mutations

| Technology | Assay status | Relative sensitivity | *KIT* mutations that can be detected | Sample input | Useful for ISM diagnosis? |
|---|---|---|---|---|---|
| **Next-generation sequencing** | Commercial use | ~200x less sensitive than ddPCR[21] | Multiple exon 17 mutations | Isolated DNA from blood or bone marrow aspirate | Only detects *KIT* D816V in ~30% of patients[22] |
| **ddPCR** | | **Current gold standard**<br>LOD: 0.022%[18] | D816V only | | Positive in ~85% of patients[18] |
| **RCA-based assay** | Research use | At least ~30x more sensitive than ddPCR[a] (this poster) | D816V only | | Positive in >95% of patients (this poster) |

[a]Lower LOD still to be validated.
ddPCR, droplet digital polymerase chain reaction; LOD, limit of detection; RCA, rolling circle amplification.

- However, even with the use of ddPCR, detection of the *KIT* D816V mutation in the peripheral blood of patients with ISM and low mast cell burden can be challenging, potentially leading to diagnostic delays and prolonged disease burden
- Diagnostic delays may be further exacerbated as another minor diagnostic criterion, serum tryptase level >20 ng/mL, may be absent in up to 30% of patients with ISM[13,14,17]
- Therefore, more sensitive assays are needed to help physicians identify patients with the *KIT* D816V mutation and underlying clonal mast cell disorders, including ISM
- superRCA® (Rarity Bioscience, Uppsala, Sweden), a rolling circle amplification technology, has been used to specifically detect *KIT* D816V mutations, with ~30-fold greater sensitivity for *KIT* D816V mutation detection *versus* ddPCR
- We used whole blood DNA samples from patients with ISM enrolled in the PIONEER study (NCT03731260) to investigate whether superRCA could detect *KIT* D816V in patients without detectable mutations by ddPCR
  - The randomized, double-blind PIONEER study (**Figure 2**) showed the efficacy of avapritinib, a potent, oral inhibitor that targets the *KIT* D816V mutation, compared with placebo and best supportive care in patients with ISM[18]
  - Avapritinib is approved in the USA and Europe for the treatment of adults with ISM, in the USA for adults with advanced SM (AdvSM), and in Europe for adults with AdvSM after ≥1 prior systemic therapy[19,20]

### Figure 2. PIONEER study design




BSC, best supportive care; ISM-SAF, Indolent Systemic Mastocytosis Symptom Assessment Form; QD, once daily; QoL, quality of life; RP2D, recommended phase 2 dose; TSS, total symptom score.

## Methods

- All patients enrolled in PIONEER met ISM diagnostic criteria, confirmed by central pathology review
- During screening, peripheral blood from all patients (N=246) was tested for the *KIT* D816V mutation by ddPCR; those without detectable mutations were retrospectively tested by superRCA
- Total symptom score (TSS) per the ISM-Symptom Assessment Form (ISM-SAF; ©2018 Blueprint Medicines Corporation) and serum tryptase levels were assessed at baseline and after 24 weeks of avapritinib therapy
  - ISM-SAF is a validated symptom assessment tool specifically developed for evaluation of ISM symptomology based on self-reported severity of 11 ISM symptoms. Scores range from 0 to 110[23,24]

### *superRCA assay*

- superRCA is a sensitive method of mutation detection that relies on DNA amplification and flow cytometry to detect low abundance mutations
- The superRCA assay is reported to detect one mutant copy out of 100,000 wild-type DNA copies[25]
- A clear separation has been reported between the range of negative DNA controls that have been tested with superRCA *versus* positive patient samples and diluted positive cell lines

## Results

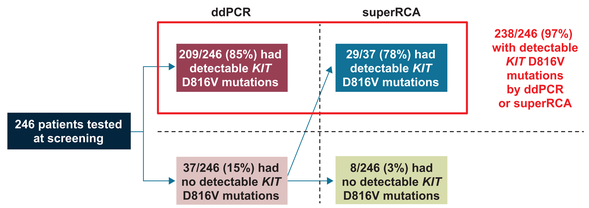
- Baseline characteristics can be found in **Table 2**
- Baseline bone marrow mast cells were significantly lower in patients with ddPCR-undetectable, superRCA-detectable *KIT* D816V mutations compared with patients with ddPCR-detectable *KIT* mutations only

### Table 2. Baseline characteristics

| Characteristic | *KIT* mutation detectable by ddPCR (n=209) | *KIT* mutation not detectable by ddPCR but detectable by superRCA (n=29) | P-value |
|---|---|---|---|
| **Age, years (range)** | 51 (18–79) | 50 (27–64) | 0.3269 |
| **Female, %** | 153 (73) | 20 (69) | 0.6586 |
| **Median baseline serum tryptase, ng/mL (range)** | 43.1 (4.2–590.4) | 20.2 (3.6–200) | <0.0001 |
| **Median BM MC, % (range)** | 10.0 (1.0–60.0) | 5.0 (1.0–40.0) | <0.0001 |
| **Mean TSS per the ISM-SAF (SD)** | 49.1 (19.6) | 48.9 (18.9) | 0.9514 |

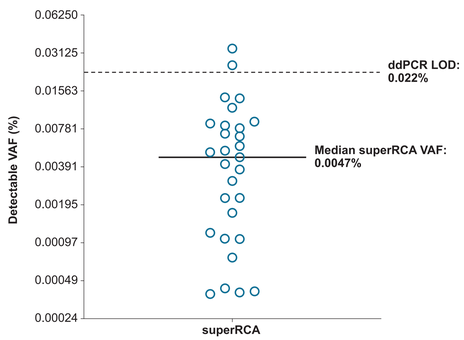
BM MC, bone marrow mast cell; SD, standard deviation.

### Figure 3. *KIT* D816V mutations detected in patients enrolled in PIONEER




- In combination, superRCA and ddPCR detected *KIT* D816V mutations in the peripheral blood of 238/246 (97%) patients (**Figure 3**)
  - Using ddPCR, *KIT* D816V was detected in 209/246 (85%) peripheral blood samples
  - In patients with ISM who had undetectable *KIT* D816V by ddPCR, superRCA detected *KIT* D816V in 29/37 (78%) samples
- The range of detectable variant allele frequencies by superRCA was 0.00038–0.03389% with a median of 0.0047% (**Figure 4**)

### Figure 4. *KIT* D816V VAF distribution by superRCA in ddPCR-undetectable patients

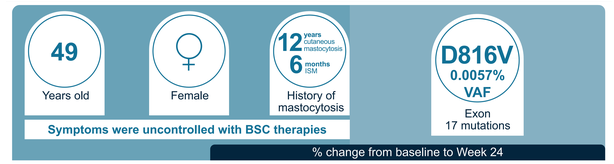



VAF, variant allele frequency.

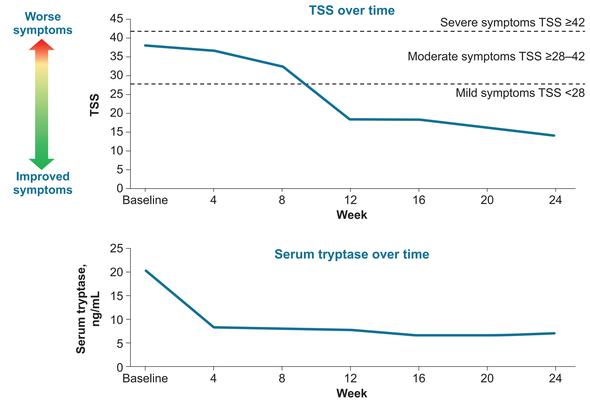
### *TSS and serum tryptase measures*

- Baseline tryptase levels were significantly lower in patients with ddPCR-undetectable, superRCA-detectable *KIT* D816V mutations compared with patients with ddPCR-detectable mutations (**Table 2**)
- Baseline symptom burden, as measured by TSS per the ISM-SAF, was similar in patients with ddPCR-detected *versus* superRCA-detected *KIT* D816V (**Table 2**)
- After 24 weeks of therapy with avapritinib, patients with detectable *KIT* D816V mutations by superRCA, but undetectable *KIT* mutations by ddPCR, had improvements in ISM-SAF TSS and tryptase, with mean percentage changes from baseline of −20.7% and −55.6%, respectively

### Case study: Effectiveness of avapritinib in a patient with a *KIT* D816V mutation below the VAF detection threshold of ddPCR, but detectable by superRCA




| | % change from baseline to Week 24 |
|---|---|
| **ISM-SAF TSS** | **−63%** |
| Skin domain score | −64% |
| Gastrointestinal domain score | −64% |
| Neuro domain score | −60% |
| **Serum tryptase** | **−65%** |




## Conclusions

- More sensitive assays are needed to aid clinicians in identifying *KIT* D816V mutations, an important minor diagnostic criterion for SM
- *KIT* D816V mutations were detected in 97% of patients when combining ddPCR and superRCA-based assay findings
  - In patients with ISM who had undetectable *KIT* D816V by ddPCR, superRCA detected *KIT* D816V in 78%
- The superRCA assay increased sensitivity limits for mutant-*KIT* D816V in peripheral blood and would facilitate the diagnosis of ISM in patients without detectable *KIT* D816V by currently commercially available tests
- A high symptom burden can still be experienced by patients without detectable *KIT* D816V by ddPCR
- Avapritinib can effectively reduce symptoms and serum tryptase levels in patients who do not have detectable *KIT* D816V by ddPCR
- Further development of superRCA may improve the detection of clonal mast cell disorders
- Until such research-based tests are widely available, bone marrow biopsy should still be performed and remains the standard-of-care for evaluating SM

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### Conflicts of interest/disclosures

Dr George has received consulting fees and is a study steering committee member for Blueprint Medicines Corporation, BMS/Celgene, Cogent Biosciences, and Incyte.
For all author disclosures, please contact medinfo@blueprintmedicines.com.

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