Ultra-Sensitive SuperRCA Assay Enhances the Ability to Detect *KIT* D816V Mutations in Peripheral Blood With a Sensitivity Comparable to That of Bone Marrow Testing

Poster Number 524

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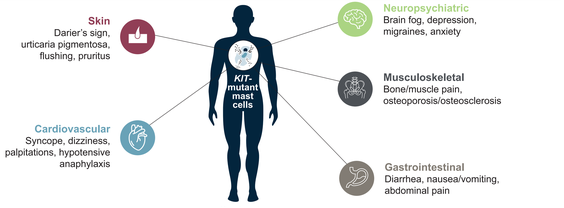
Introduction

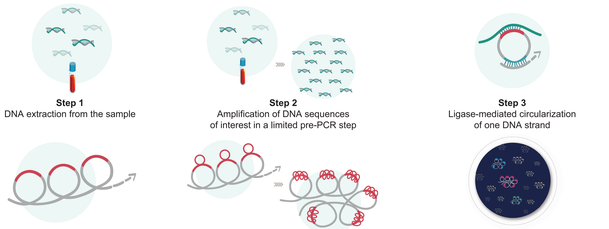
- Indolent systemic mastocytosis (ISM) is a clonal mast cell disease driven by the *KIT* D816V mutation in ~95% of patients[1–4]
- The prevalence of systemic mastocytosis (SM) has been estimated at up to 1 in 5000 people, and ISM is the most common subtype of SM[5–8]
- ISM is characterized by long-term, debilitating cutaneous, gastrointestinal, neurological, and musculoskeletal symptoms[9–13] (**Figure 1**)
- The detection of *KIT* D816V mutation is one of the four minor diagnostic criteria for SM[14,15]
- Droplet digital polymerase chain reaction (ddPCR) is the current gold standard test for the detection of the *KIT* D816V mutation;[16] however, false-negatives for peripheral blood (PB) *KIT* D816V in patients with ISM can occur due to low mutation burden (low circulating variant allele frequency) and assay sensitivity limits (limit of detection [LoD] of commercial ddPCR: 0.03%),[17] potentially leading to diagnostic and treatment delays
- The ultra-sensitive *KIT* D816V super rolling-circle amplification (superRCA) assay that uses flow cytometry readout was developed using superRCA technology (superRCA®; Rarity Bioscience, Uppsala, Sweden) to specifically detect *KIT* D816V with ≥30-fold greater sensitivity (LoD <0.001%) *versus* ddPCR[18] (**Figure 2**) and had 100% concordance of with the ddPCR assay[19]
- In this study, we compared *KIT* D816V detection with each method and baseline characteristics for ddPCR-detectable *versus* ddPCR-undetable/superRCA-detectable patients with ISM
  - Patients were enrolled in PIONEER (NCT03731260) and HARBOR (NCT04773782) studies of selective KIT D816V inhibitors avapritinib (approved for ISM[20,21]) and investigational elenestinib

Figure 1. ISM symptoms



ISM, indolent systemic mastocytosis.

Figure 2. SuperRCA® methodology



DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; RCA, rolling-circle amplification; superRCA, super rolling-circle amplification.

Methods

- All patients enrolled in PIONEER and HARBOR met ISM diagnostic criteria, confirmed by central pathology review
- PB *KIT* D816V detectability from PIONEER/HARBOR patients (assessed centrally during screening by ddPCR [LoD: PIONEER, 0.022%; HARBOR, 0.03%] and retrospectively on ddPCR-undetectable patients using superRCA) was compared to bone marrow (BM) aspirate *KIT* D816V detectability (assessed locally)
- Total Symptom Score (TSS) per the ISM-Symptom Assessment Form (ISM-SAF; ©2018 Blueprint Medicines Corporation) is a validated symptom assessment tool specifically developed for evaluation of ISM symptomology based on self-reported severity of 11 ISM symptoms (score severity increases across a range of 0–110)[22]
- We compared baseline symptom burden using ISM-SAF TSS, and objective measures of mast cell burden (serum tryptase levels and bone marrow mast cells [BM MCs]) between ddPCR-detectable *versus* ddPCR-undetectable/superRCA-detectable patients

Results

PIONEER/HARBOR Combined

- Of 376 patients, 80% (300/376) had *KIT* D816V detectable by ddPCR
  - Combining ddPCR and ultra-sensitive *KIT* D816V superRCA assay data increased PB *KIT* D816V detection to 95% of all patients
- Concordance with local BM *KIT* D816V positivity was observed in 97% of patients across HARBOR and PIONEER with local BM *KIT* D816V assessments

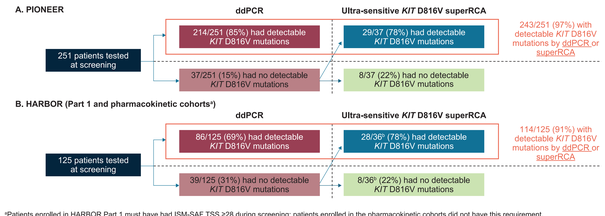
PIONEER

- Ultra-sensitive *KIT* D816V superRCA assay and ddPCR detected *KIT* D816V mutations in the PB of 243/251 (97%) of patients (**Figure 3A**)
  - Using ddPCR alone, *KIT* D816V was detected in 214/251 (85%) PB samples
  - Of the 37 ddPCR *KIT* D816V–undetected samples, the mutation was detected by ultra-sensitive *KIT* D816V superRCA assay in 29 samples (78%) in PB
- The range of detectable variant allele frequencies by ultra-sensitive *KIT* D816V superRCA assay was 0.00038–0.03389% with a median of 0.0047% (**Figure 4A**)

HARBOR (Part 1 and pharmacokinetic cohorts)

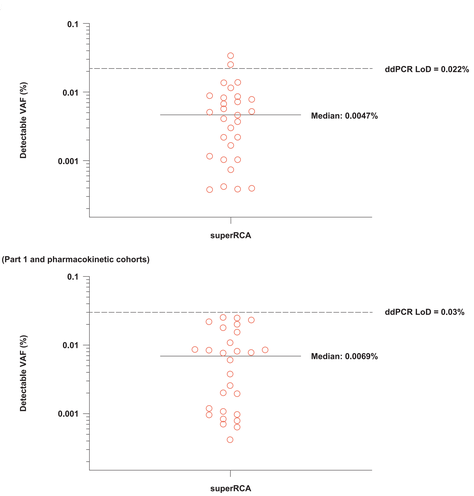
- Ultra-sensitive *KIT* D816V superRCA assay and ddPCR detected *KIT* D816V mutations in the PB of 114/125 (91%) of patients (**Figure 3B**)
  - Using ddPCR alone, *KIT* D816V was detected in 86/125 (69%) PB samples
  - Of the 39 samples that did not have *KIT* D816V detected by ddPCR, 36 were evaluated by ultra-sensitive *KIT* D816V superRCA assay and 28 of these (78%) had detectable *KIT* D816V in PB (three samples were not tested by superRCA)
- The range of detectable variant allele frequencies by ultra-sensitive *KIT* D816V superRCA assay was 0.00040–0.02550% with a median of 0.0069% (**Figure 4B**)

Figure 3. *KIT* D816V mutations detected in peripheral blood from patients enrolled in PIONEER (A) and HARBOR (B)



[a]Patients enrolled in HARBOR Part 1 must have had ISM-SAF TSS ≥28 during screening; patients enrolled in the pharmacokinetic cohorts did not have this requirement.
[b]Three samples were not evaluated by superRCA.
ddPCR, droplet digital polymerase chain reaction; ISM-SAF TSS, Indolent Systemic Mastocytosis–Symptom Assessment Form Total Symptom Score.

Figure 4. *KIT* D816V VAF distribution by ultra-sensitive *KIT* D816V superRCA assay in ddPCR-undetectable patients enrolled in PIONEER (A) and HARBOR (B)



LoD, limit of detection; VAF, variant allele frequency.

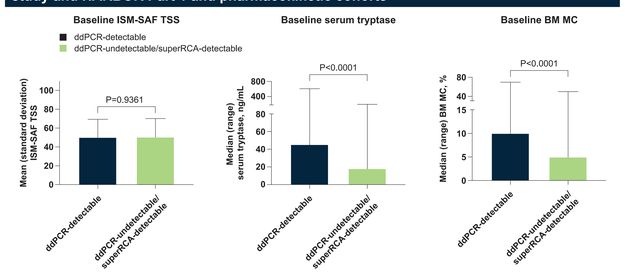
- Baseline characteristics for combined patients from the PIONEER and HARBOR studies are presented in **Table 1**
- ISM-SAF TSS score was not different between detection groups (**Table 1**)
- Baseline serum tryptase and BM MCs were significantly lower in patients with ddPCR-undetectable/superRCA-detectable *KIT* D816V mutations compared to patients with ddPCR-detectable mutations (**Table 1** and **Figure 5**)

Table 1. Demographics of patients with ISM enrolled in PIONEER and HARBOR studies for those who were ddPCR-detectable and ddPCR-undetectable/superRCA-detectable at screening

	PIONEER and HARBOR (Part 1 and pharmacokinetic cohort) combined		
Avapritinib	ddPCR-detectable (n=300)	ddPCR-undetectable/ superRCA-detectable (n=57)	P-value
Characteristic			
Median (range) age, years	52 (18–79)	50 (23–72)	0.0510
Female, %	220 (73)	41 (72)	0.8708
Mean (SD) ISM-SAF TSS	49.6 (19.5)	49.9 (20.0)	0.9361
Median (range) baseline serum tryptase, ng/mL	45.2 (4.2–612.0)	17.7 (3.6–200.0)	<0.0001
Median (range) BM MC, %	10.0 (1.0–70.0)	5.0 (1.0–50.0)	<0.0001

BM MC, bone marrow mast cell; RCA, rolling-circle amplification; SD, standard deviation; TSS, total symptom score.

Figure 5. Combined baseline symptom burden (ISM-SAF TSS) and objective measures of disease burden (serum tryptase and BM MCs) for patients enrolled in the PIONEER study and HARBOR Part 1 and pharmacokinetic cohorts



Conclusions

- The current gold standard, ddPCR assay, fails to detect the *KIT* D816V mutation in PB of a significant number of patients, and bone marrow aspirate *KIT* testing is not always feasible
- The ultra-sensitive *KIT* D816V superRCA assay detected a *KIT* D816V mutation in 78% of samples where a *KIT* D816V mutation could not be detected by ddPCR. Together, these assays increased the detection rate of *KIT* D816V in PB to 95% of the ISM population (*vs* 80% by ddPCR alone). Together, these assays also detected a *KIT* D816V mutation in 97% of patients that had detectable *KIT* D816V by local BM testing
- Patients with *KIT* D816V detected by ultra-sensitive superRCA had lower baseline tryptase and BM MCs than patients with *KIT* D816V detected by ddPCR, but similar symptom scores, highlighting that patients can still suffer from a high symptom burden even with lower MC burden
- Confident early detection of patients with ISM with high sensitivity is essential to direct effective treatment
- Although ultra-sensitive *KIT* D816V superRCA testing of PB in this study had high concordance with BM testing, BM biopsy should still be performed under current diagnostic criteria and testing availability, especially in patients with a high clinical index of suspicion despite negative PB *KIT* testing

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Conflicts of interest/disclosures

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