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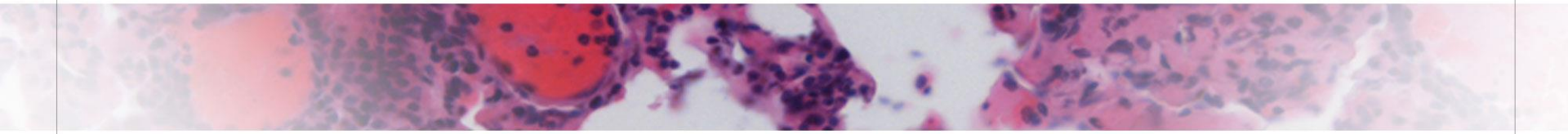

Increased detection of *KIT* D816V mutation in peripheral blood samples from patients with indolent systemic mastocytosis (ISM) in the phase 2 PIONEER study using a high sensitivity droplet digital (dd) PCR assay compared with next generation sequencing (NGS)

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Disclosures

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Study sponsored by Blueprint Medicines Corporation.

AYVAKIT™ (avapritinib) is approved by the US Food and Drug Administration (FDA) for the treatment of adults with unresectable or metastatic gastrointestinal stromal tumor (GIST) harboring a platelet-derived growth factor receptor alpha (*PDGFRA*) exon 18 mutation, including *PDGFRA* D842V mutations.

In Europe, AYVAKYT® (avapritinib) is approved by the European Medicines Agency (EMA) for the treatment of adult patients with unresectable or metastatic gastrointestinal GIST harboring the *PDGFRA* D842V mutation.

Avapritinib is not approved as safe or effective for use in systemic mastocytosis or any other indication by the FDA, EMA, or any healthcare authority in any jurisdiction.

Background and methods

- SM is a rare, clonal 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]
- NGS often lacks sufficient sensitivity to detect *KIT* D816V, leading to potential misdiagnosis
- A highly sensitive assay[6] such as ddPCR is essential to reliably detect the *KIT* D816V MAF, especially in PB samples[7]

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

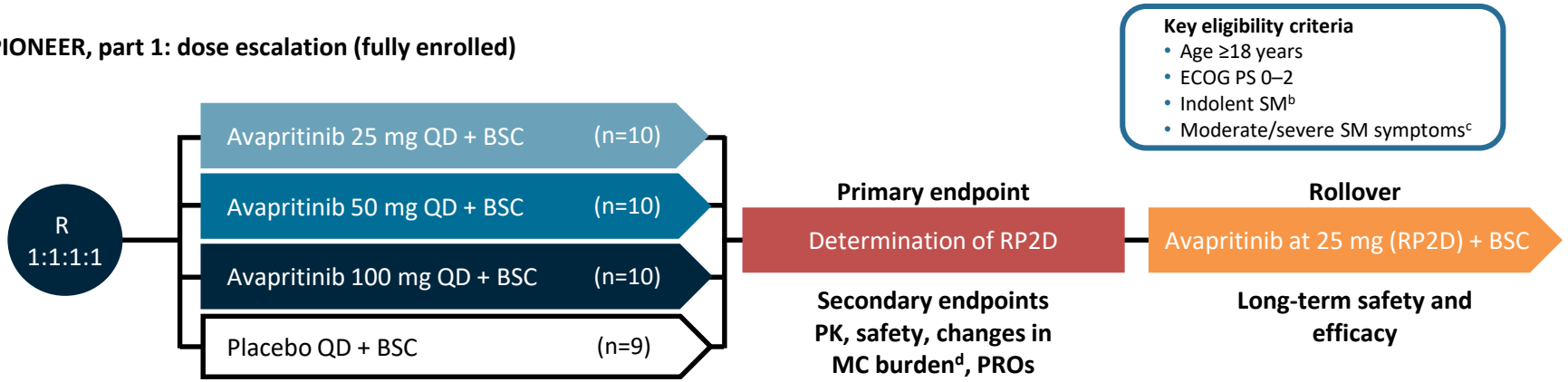
[a]In the BM or another extracutaneous organ. BM, bone marrow; ddPCR, droplet digital polymerase chain reaction; MC, mast cell; NGS, next-generation sequencing; PB, peripheral blood; SM, systemic mastocytosis; MAF, mutation allele fraction.

1. Gulen T et al. *J Intern Med.* 2016;279:211–228; 2. Pardanani A. *Am J Hematol.* 2016;91:1146–1159; 3. Kristensen T et al. J *Mol Diagn*. 2011:180–188; 4 Valent P et al. *Blood*. 2017;129:1420–1427; 5. Jennings SV et al. *Immunol Allergy Clin North Am*. 2018;38:505–525; 6. Reiter A et al. *Blood*. 2020;135:1365–1376; 7. Greiner G et al. *Haematologica*. 2020;105:366–374.

Study design

- Central ddPCR (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
  - 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

PIONEER, part 1: dose escalation (fully enrolled)



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

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	All doses (n=39)		
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.

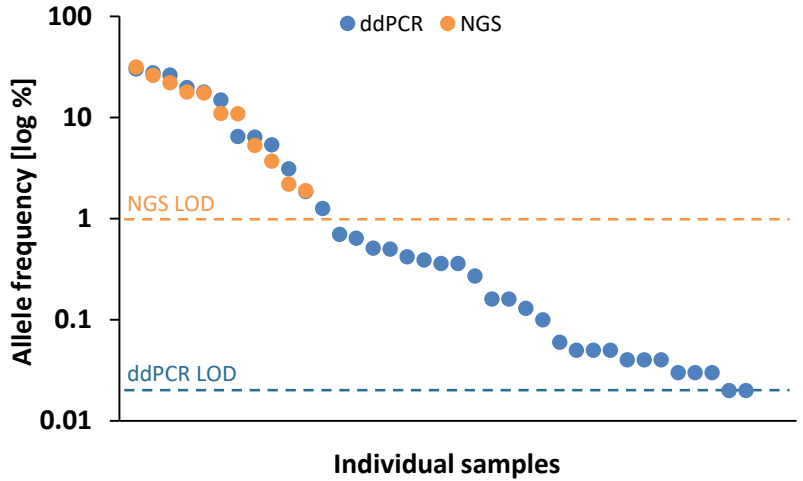
Confirmed diagnostic characteristics of enrolled patients

	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	8 (89)	7 (70)	8 (80)	7 (70)	30 (77)
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.

Performance of central ddPCR and NGS detection of *KIT* D816V MAF

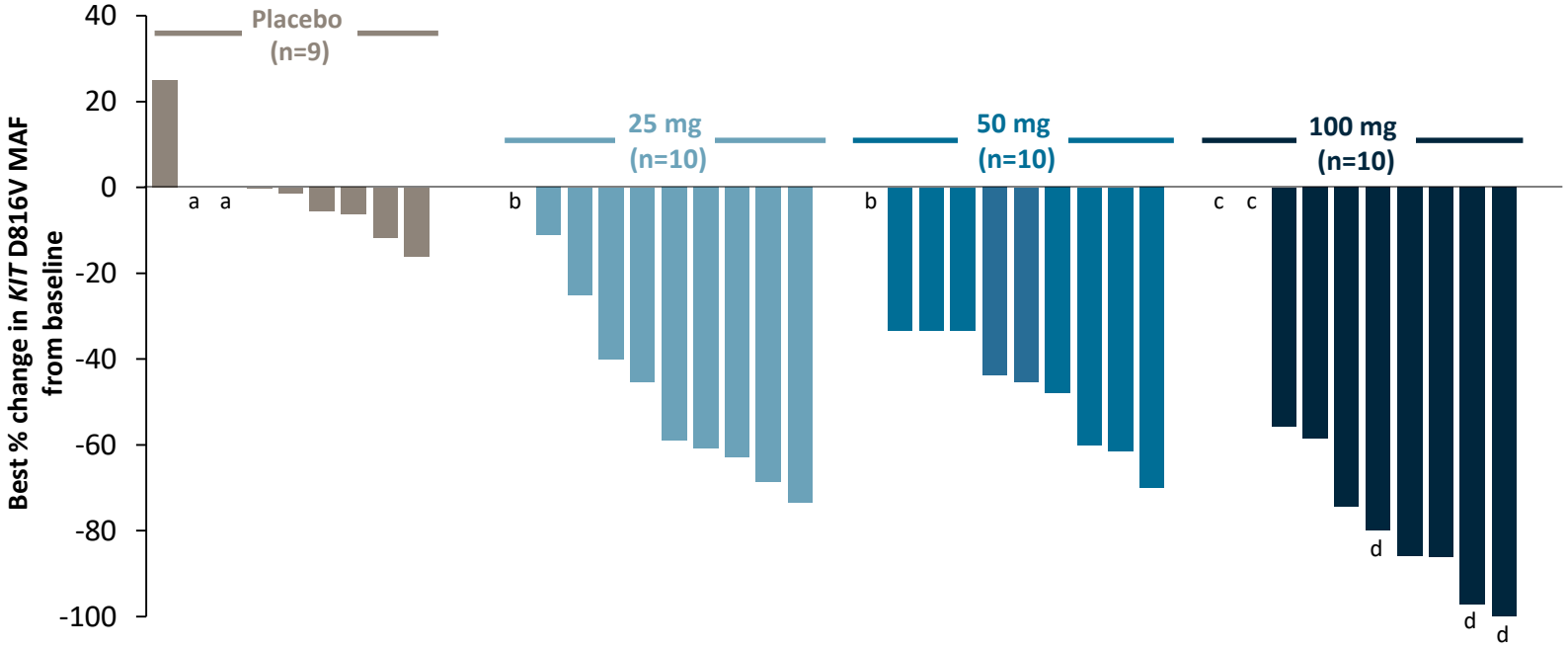
	Local assessment n (%)	TruSight NGS n (%)	ddPCR n (%)
KIT D816V detected	31 (80)	11 (28)	37 (95)
KIT D816V not detected	8 (20)	28 (72)	2 (5)
Patients analyzed	39	39	39



- The high-sensitivity ddPCR assay method demonstrated:
  - *KIT* D816V mutation detection in 95% of PB samples from patients with previously confirmed ISM
  - 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
  - Greater diagnostic sensitivity for ISM compared with serum tryptase >20 ng/mL (77%) and presence of BM MC aggregates (90%)

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 screening or C1D1 were plotted for the scatter graph.

Objective reductions in *KIT* D816V MAF 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 ISM patients
- 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 be also be used to therapeutically monitor ISM patients during the course of therapy with highly specific tyrosine kinase inhibitors, such as avapritinib

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