Responses to Avapritinib in Patients Without Detectable *KIT* Mutations by ddPCR in Peripheral Blood Highlight Diagnostic Challenges and Opportunities in Indolent Systemic Mastocytosis

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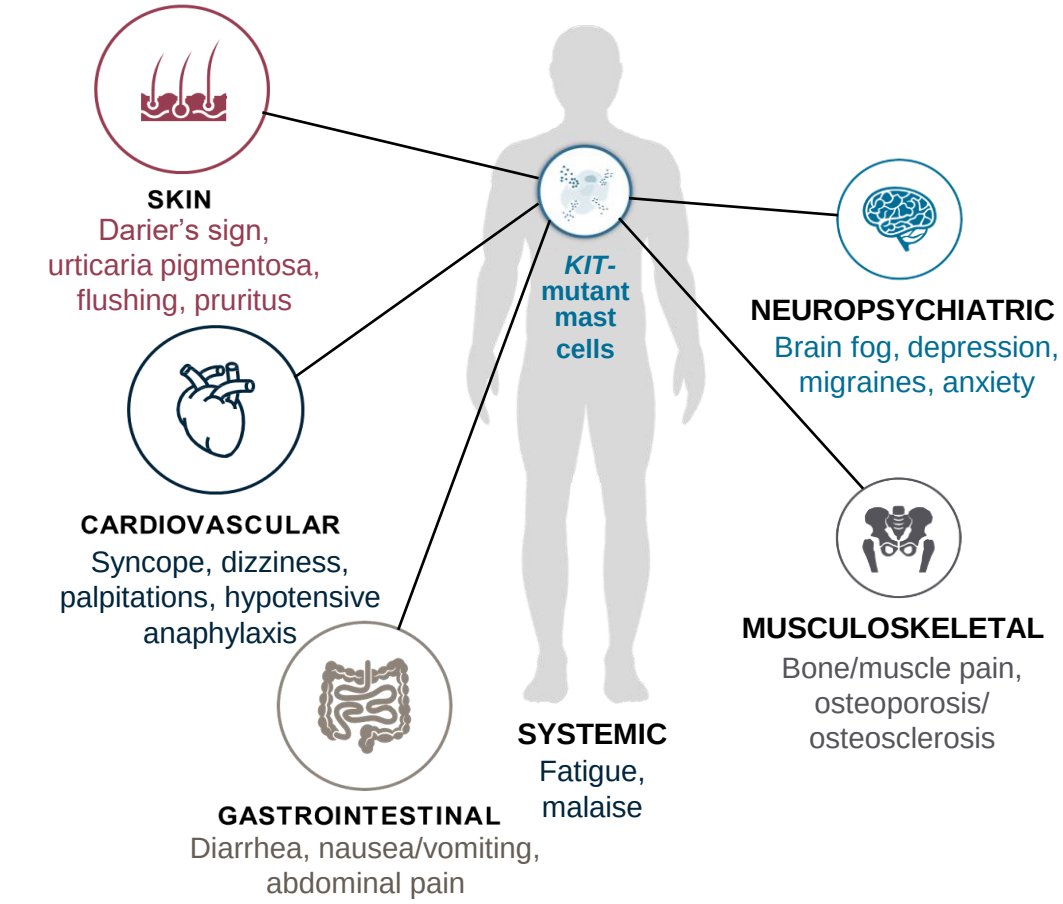
Indolent systemic mastocytosis: A *KIT* D816V mutation–driven disease with substantial impact on quality of life

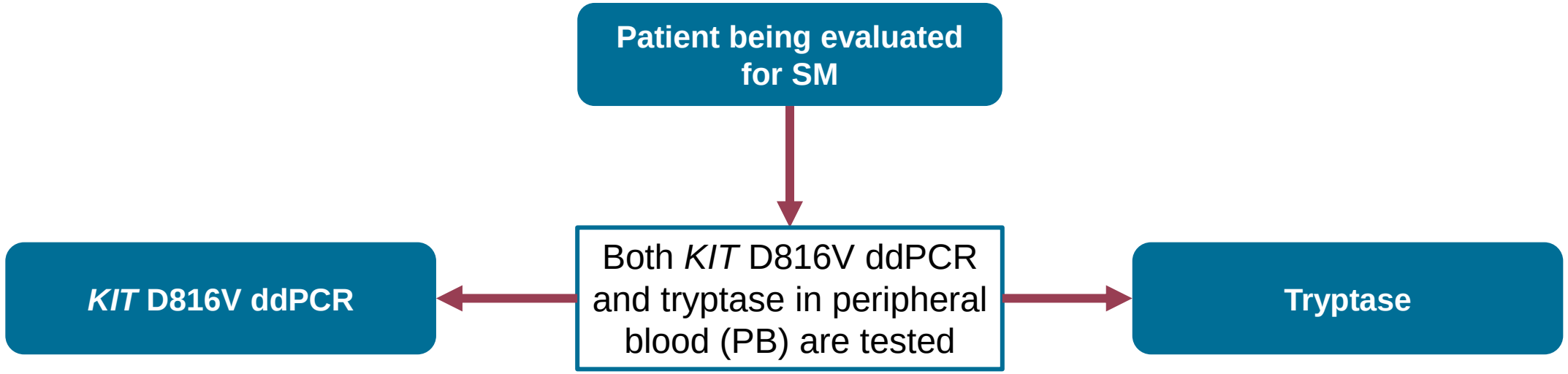
- **Indolent systemic mastocytosis** (ISM), the **most common** subtype of systemic mastocytosis (SM), is driven by aberrant mast cells carrying a *KIT* D816V mutation in ~95% of cases[1–3]
- The diagnosis of SM is made according to a set of criteria defined by expert consensus[8–10]
- One of the diagnostic criteria is demonstrating the presence of a *KIT* mutation
  - *KIT* mutations can be difficult to detect in blood due to low levels of circulating KIT-mutant cells in ISM
  - SM cannot be ruled out if ddPCR does not detect a mutation in blood: a bone marrow biopsy is still required if suspicion is high

Patients with ISM can have lifelong **debilitating symptoms** across multiple organ systems[4–7]



ISM, indolent systemic mastocytosis.

Peripheral blood testing in a patient with suspected SM has advantages but could be improved



Advantages

- Samples easily collected (blood draw), tested, and interpreted
- Droplet digital polymerase chain reaction (ddPCR) has the highest sensitivity for *KIT* D816V mutation out of the current clinically available tests (much higher than next generation sequencing)[11]
- Presence of *KIT* activating mutation is a minor diagnostic criterion for SM

Disadvantages

- Imperfect sensitivity: only 85% of patients with ISM were detected in the PIONEER study[12]
- Cannot be used as a “rule-out” test, bone marrow biopsy is still required if suspicion is high

Advantages

- Samples easily collected and tested (blood draw)
- Tryptase levels >20 ng/mL is a minor diagnostic criterion for SM[9]

Disadvantages

- Interpretation can be challenging, hereditary alpha tryptasemia status is needed
- Poor sensitivity, as up to 30% of patients with SM may have tryptase <20 ng/mL[13]
- Cannot rule out SM even if tryptase levels <20 ng/mL

ddPCR, droplet digital polymerise chain reaction; SM, systemic mastocytosis.

Detection methods for *KIT* mutations in SM vary in sensitivity: ddPCR is the current gold standard and more sensitive than NGS

Technology	Assay status	LOD for *KIT* D816V mutations	*KIT* mutations that can be detected	Sample input	Useful for ISM diagnosis?
NGS	Commercial use	5%[11]	Multiple exon 17 mutations	Isolated DNA from blood or bone marrow aspirate	Only detects *KIT* D816V in ~30% of patients[14]
ddPCR		0.022%[12]	D816V only		**Current gold standard,** positive in ~85% of patients[12]
Duplex sequencing	Research use	0.0013%[16]	Multiple exon 17 mutations		**17x more sensitive than ddPCR**

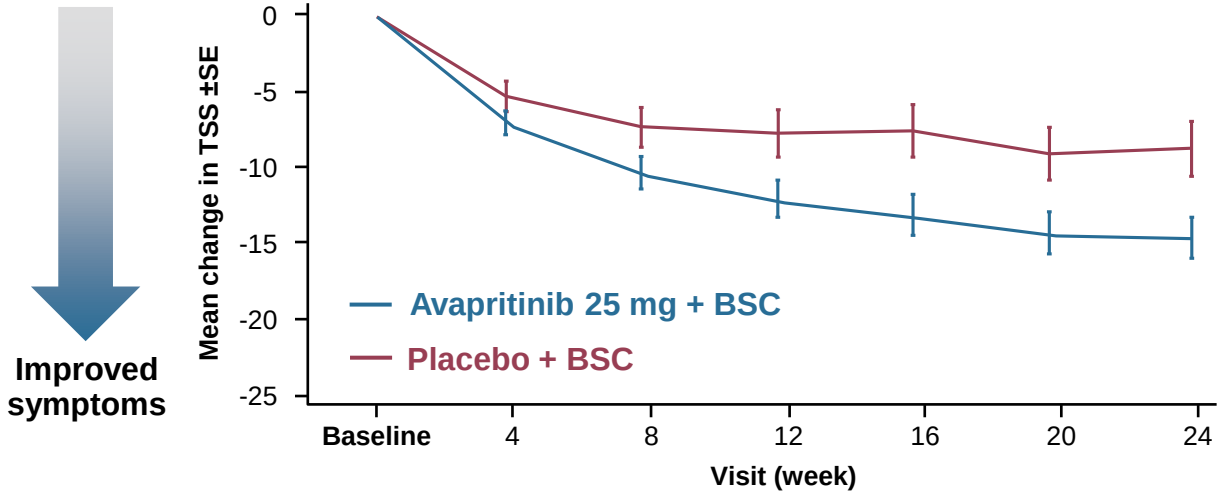
To determine whether ultra-sensitive duplex sequencing facilitates detection of more *KIT* mutations, we evaluated its use on clinical trial samples from patients with verified ISM who had no detectable *KIT* mutation by ddPCR

LOD, limit of detection; NGS, next-generation sequencing.

The cohort of patients enrolled in the PIONEER trial of avapritinib represents an opportunity to better understand ISM

- PIONEER (NCT03731260) is a double-blind, placebo-controlled trial of avapritinib in patients with ISM[12]
- Avapritinib potently and selectively inhibits KIT D816V[17]
- In PIONEER, avapritinib significantly improved total symptom score (TSS) as assessed by the ISM-Symptom Assessment Form[a] (ISM-SAF),[12] leading to approval for adults with ISM in the USA and Europe[18,19]
- PIONEER required ddPCR testing for *KIT* D816V mutations in all patients at time of enrollment

Mean change in ISM-SAF TSS over time



Number of patients

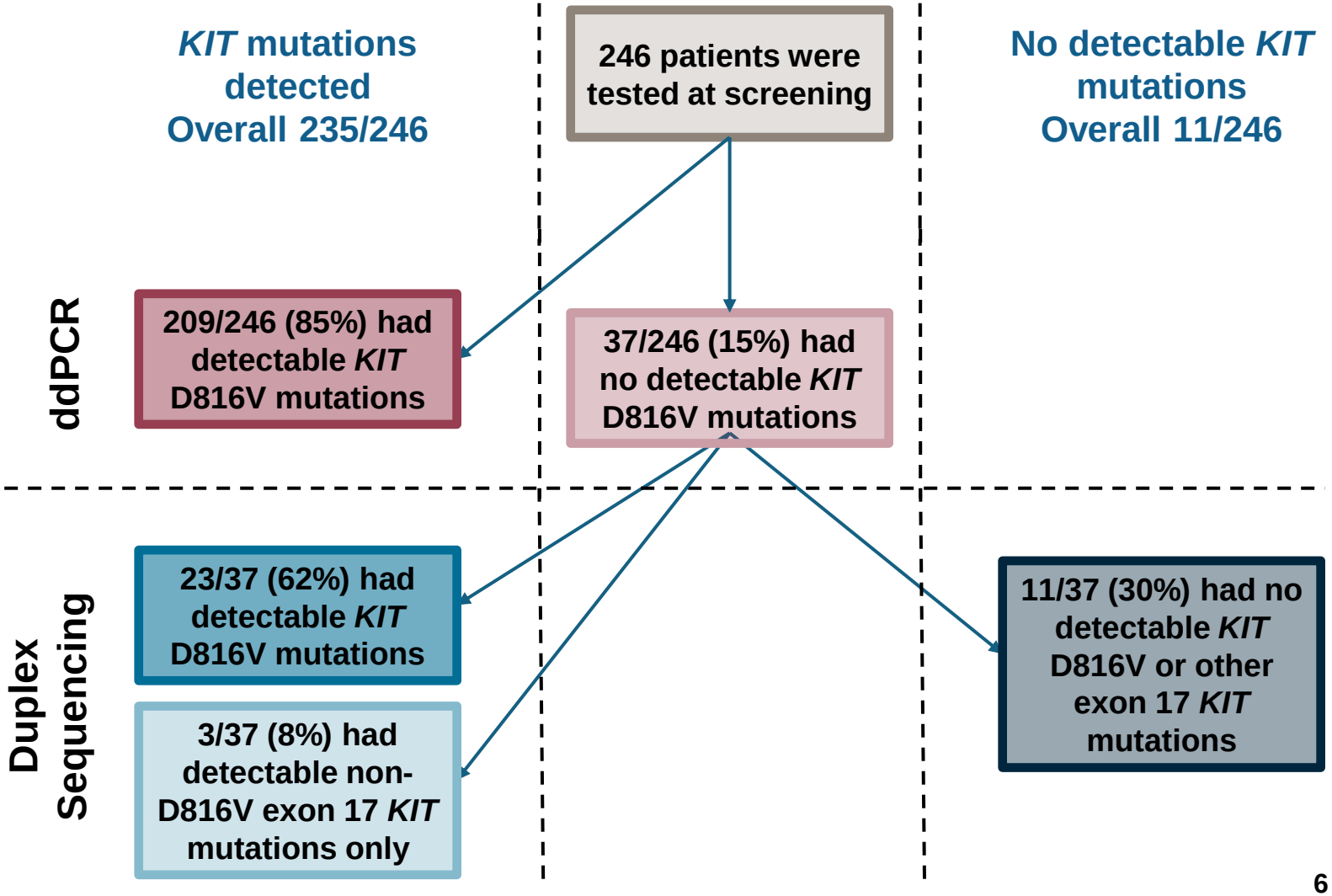
Avapritinib + BSC	139	137	135	135	137	136	133
Placebo + BSC	71	71	71	68	67	66	66

Use of patient samples from PIONEER allowed avapritinib response assessment in patients who did not have detectable *KIT* D816V mutations by ddPCR

[a]ISM-SAF © 2018 Blueprint Medicines Corporation. BSC, best supportive care; SE, standard error.

Patients who enrolled in PIONEER had peripheral blood testing for *KIT* mutations at screening and subdivided into groups

- Patients who had no detectable *KIT* D816V in PB by ddPCR were further tested with duplex sequencing
- Of 37 patients with no detectable *KIT* D816V by ddPCR, 26 had activating *KIT* mutations detectable by duplex sequencing
- Combining results from clinical ddPCR testing and research duplex sequencing, **96% of patients from PIONEER had detectable activating *KIT* mutations**



Patients with *KIT* mutations detectable only by duplex sequencing had a lower baseline disease burden

Characteristic	*KIT* mutation detectable by ddPCR (n=209)	*KIT* mutation not detectable by ddPCR and detectable by duplex sequencing (n=26)	P-value
Age, years (range)	51 (18–79)	48 (31–64)	0.24
Female, %	153 (73)	20 (77)	0.82
Median baseline serum tryptase, ng/mL (range)	43.1 (4.2–590.4)	23.4 (3.6–250.4)	<0.01
Median BM MC, % (range)	10 (1.0–60.0)	5.0 (1.0–40.0)	<0.001
Median *KIT* D816V VAF, % (range)	0.51 (0.02–41.3)	0.0068 (0.0013–0.0261)	<0.0001

BM, bone marrow; MC, mast cell; VAF, variant allele fraction.

Duplex sequencing also successfully identified non-canonical *KIT* mutations that cannot be detected by ddPCR

- A total of 21/26 patients had a detectable lone *KIT* D816V mutation
- Other *KIT* mutations were detected in 5/26 patients, including:
  - Patients (n=2) with dual mutations in *KIT* (D816I+D816V, C788Y+D816V)
  - Patients (n=3) with lone non-D816V *KIT* activating mutations (D816I, D816Y; VAF 0.0075–4.5%)

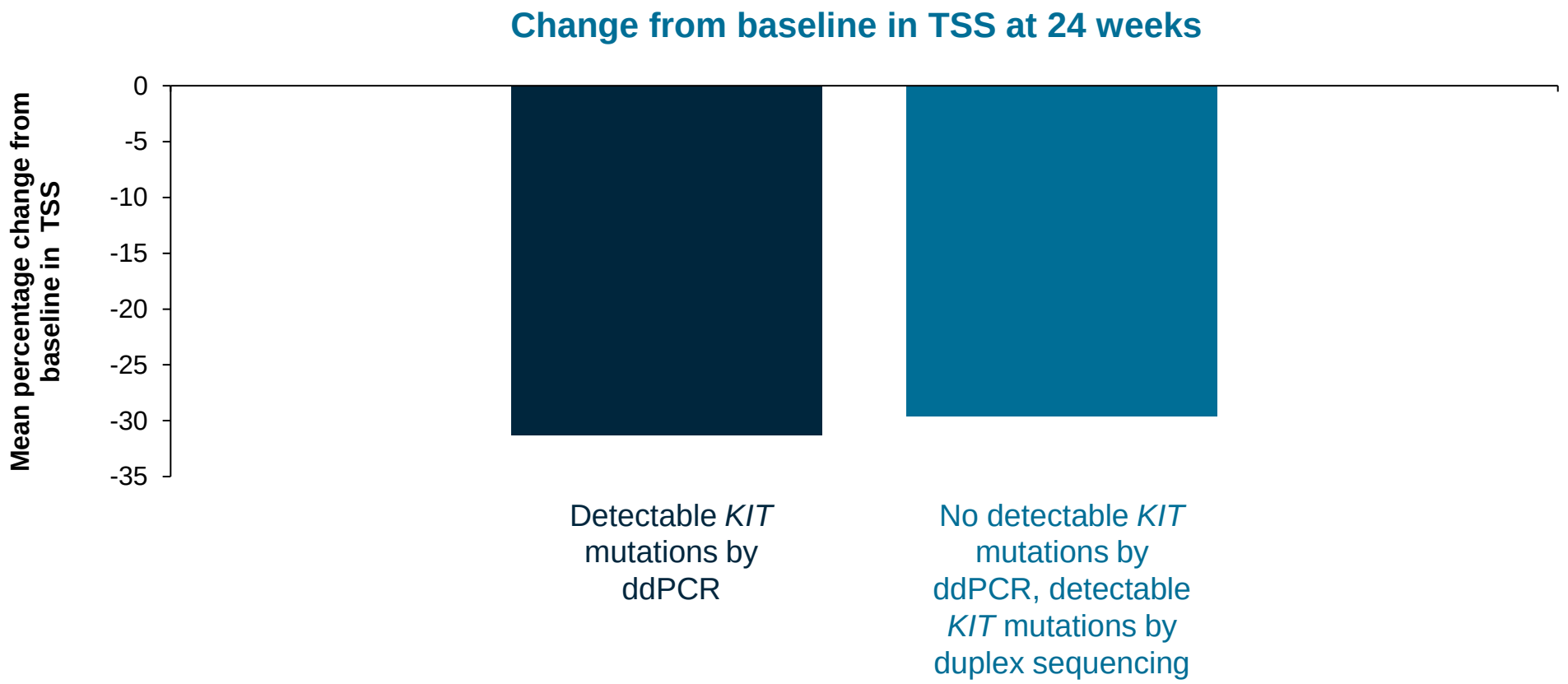
Patients with non-D816V *KIT* mutations detected by duplex sequencing

Age, years	Gender	Mutations detected	Median *KIT* mutation VAF, % by duplex sequencing	Avapritinib sensitivity *in vitro* (IC_{50} <1 nm)[18]
63	Female	D816I/D816V	0.0013/0.0026	Yes/Yes
33	Female	D816I	0.7820	Yes
52	Male	D816Y	4.4781	Yes
51	Female	D816Y	0.0075	Yes
31	Female	C788Y/D816V	0.0041/0.0037	ND/Yes

IC_{50}, half maximal inhibitory concentration. ND, not determined.

Similar improvements were seen in mean percentage change from baseline in TSS irrespective of the test used to detect *KIT* mutations

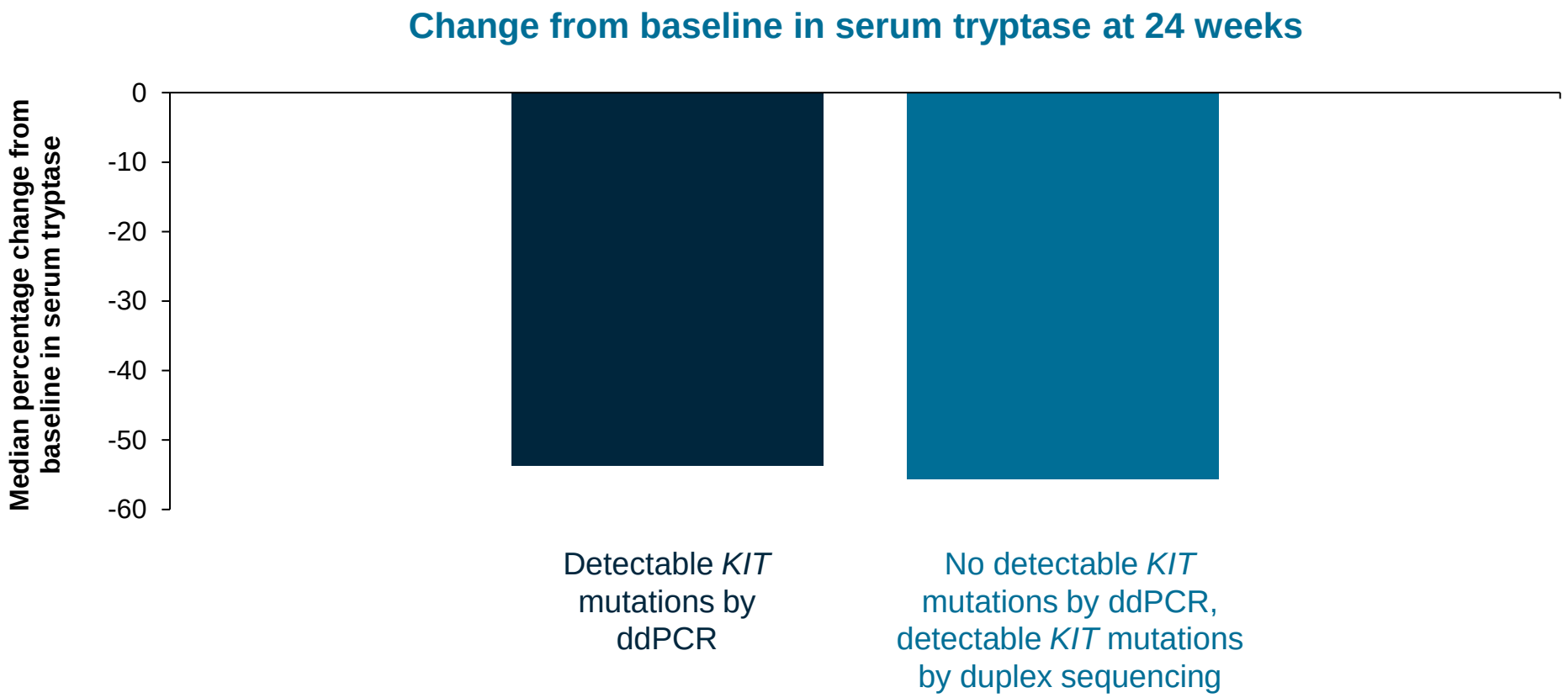
- After 24 weeks of therapy, improvements were seen for avapritinib-treated patients[a] in mean percentage change from baseline in TSS whether *KIT* mutations were detected by ddPCR (n=194) or by duplex sequencing (n=22)



[a]Patients who initiated avapritinib 50 mg (n=10) or avapritinib 100 mg (n=9) during Part 1 of the PIONEER study have not been included in assessments.

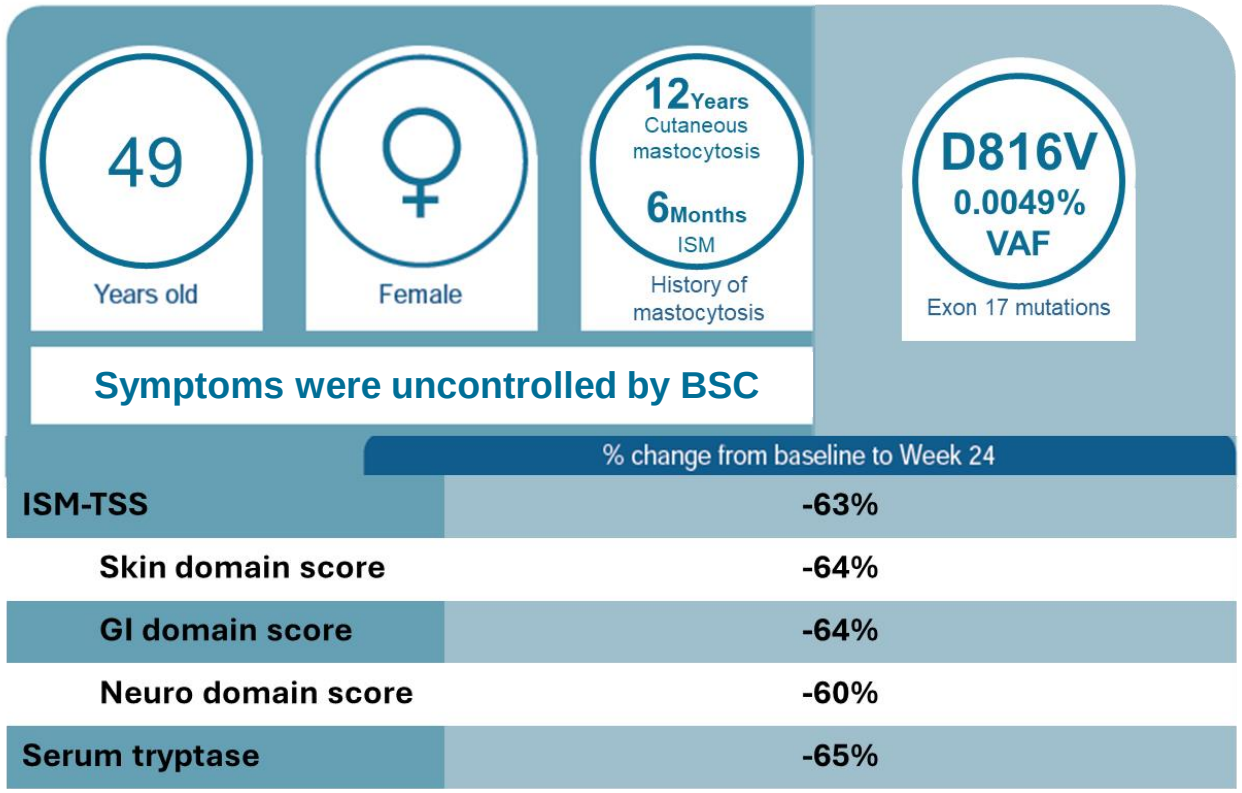
Similar median percentage change from baseline in serum tryptase levels in avapritinib-treated patients at 24 weeks by *KIT* mutational status

- After 24 weeks of avapritinib treatment, improvements were seen in tryptase percentage change from baseline in patients[a] whether *KIT* mutations were detected by ddPCR (n=194) or by duplex sequencing only (n=22)

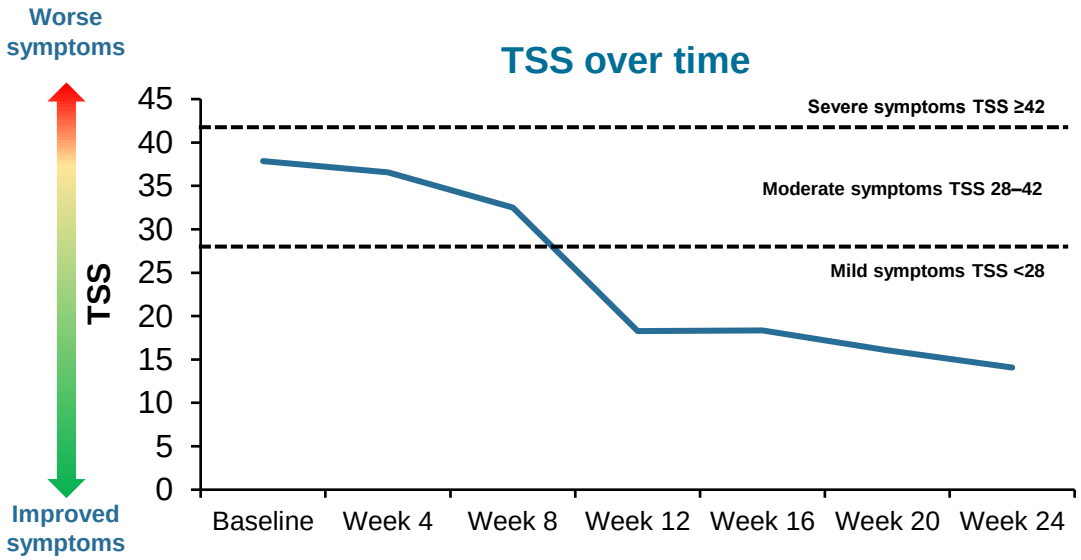


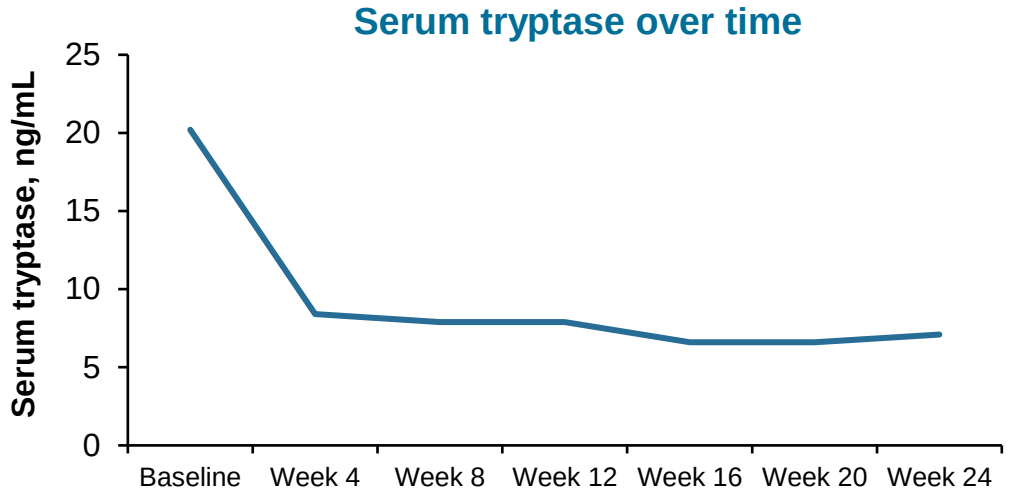
[a]Patients who initiated avapritinib 50 mg (n=10) or avapritinib 100 mg (n=9) during Part 1 of the PIONEER study have not been included in assessments.

Informative case study: Effectiveness of avapritinib in a patient with a *KIT* D816V mutation below the VAF detection threshold of ddPCR



	% change from baseline to Week 24
ISM-TSS	-63%
Skin domain score	-64%
GI domain score	-64%
Neuro domain score	-60%
Serum tryptase	-65%





GI, gastrointestinal

Conclusions

- Due to the rarity of circulating mutant cells in PB in ISM, more sensitive assays are needed to aid clinicians in identifying *KIT* D816V mutations, an important minor diagnostic criterion
- While serum tryptase and ddPCR testing for *KIT* D816V in PB are important tests in the work-up of suspected SM, the possibility of SM cannot be ruled out when *KIT* D816V is not detected
- The combination of ddPCR testing and ultra-sensitive duplex sequencing was able to identify an activating exon 17 *KIT* mutation in the blood of 96% of patients with ISM in PIONEER
  - We found that 70% of patients with undetectable *KIT* D816V by ddPCR had activating *KIT* mutations detected by duplex sequencing
- Avapritinib can effectively reduce symptoms even in patients who do not have detectable *KIT* D816V by ddPCR
- Bone marrow biopsy, including ddPCR of the bone marrow aspirate sample for *KIT* D816V, is still the standard-of-care for evaluating SM and should be performed if SM is suspected

References

1. Kristensen T, et al. *Am J Hematol*. 2014;89:493–498.
2. Ungerstedt J, et al. *Cancers*. 2022;14:3942.
3. Garcia-Montero AC, et al. *Blood*. 2006;108:2366–2372.
4. Mesa RA, et al. *Cancer*. 2022;128:3691–3699.
5. Hermine O, et al. *PLoS One*. 2008;3:e2266.
6. van Anrooij B, et al. *Allergy*. 2016;71:1585–1593.
7. Akin C, et al. *J Allergy Clin Immunol*. 2022;149:1912–1918.
8. Verstovek S. ©International Agency; for Research on Cancer. 2023. Systemic Mastocytosis. https://tumourclassification.iarc.who.int/chaptercontent/63/20. Accessed January 20, 2023.
9. Khoury JD et al. *Leukemia*. 2022;36:1703–1719.
10. Valent P et al. *Hemasphere*. 2021;5:e646.
11. TruSight™ Myeloid Sequencing Panel Data Sheet. https://www.illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/trusight-myeloid-data-sheet-m-gl-00320/trusight-myeloid-data-sheet-m-gl-00320.pdf. Accessed February 14, 2025.
12. Gotlib J et al. *NEJM Evid.* 2023;2:EVIDoa2200339
13. Sánchez-Muñoz L et al. *Mod Pathol*. 2011;24:1157–1168
14. George T et al. Presented at the American Society of Hematology Annual Meeting and Exposition, 2020; Presentation #3004.
15. Twinstrand Biosciences Technology Brochure. https://twinstrandbio.com/technology/. Accessed October 15, 2024.
16. Radia DH et al. Presented at the American Society of Hematology Annual Meeting and Exposition, 2024. Poster #3164.
17. Evans EK et al. *Sci Transl Med*. 2017;9:eaao1690.
18. Blueprint Medicines Corporation. AYVAKIT® (avapritinib). Prescribing Information. 2024. https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/212608s020lbl.pdf. Accessed December 18, 2024.
19. Blueprint Medicines Corporation. AYVAKYT® (avapritinib). Summary of Product Characteristics. 2024. https://www.ema.europa.eu/en/documents/product-information/ayvakyt-epar-product-information_en.pdf. Accessed December 18, 2024.

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Conflicts of interest and disclosures

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