Droplet Digital PCR for Non-Invasive Detection of the KIT D816V Mutation in the Peripheral Blood of patients with Suspected Systemic Mastocytosis

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Background

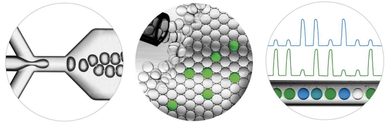
- Detection of the D816V KIT mutation in the bone marrow (BM) is one of the minor criteria for the diagnosis of Systemic Mastocytosis (SM)
- Non-invasive pre-screening of peripheral blood (PB) by ASO-qPCR has been shown to detect the D816V mutation with a hight concordance with BM results in indolent SM (ISM). However, ASO-qPCR is not standardized, and commercial kits are not available
- Droplet digital PCR (ddPCR) might be a valuable alternative to ASO-qPCR

Objective

- ➢ To validate a commercially available ddPCR assay for the detection and quantitation of the KIT D816V mutation
- ➢ To evaluate ddPCR potential as a non-invasive screening tool in patients with suspected systemic mastocytosis

Materials and Methods

Droplet digital PCR was performed using 50 ng/µL of genomic DNA with the KIT p.D816V human mutation assay on a QX200 instrument (Bio-Rad).



- PB samples from 30 healthy donors (HDs) were used to calculate the limit of blank
- The limit of detection was determined by mixing KIT D816V-mutated (HMC-1.2) and unmutated (HMC-1.1) cell lines in order to mimic different allele burdens, from 50% down to 0.01%
- Accuracy was investigated by analyzing, in parallel, 35 samples from patients with confirmed or suspected ISM by ASO-qPCR and ddPCR
- Concordance between ddPCR results in BM and PB was assessed in 60 matched PB/BM samples

Results

- No KIT D816V-positive events were detected in any of the HD samples (Figure 1)

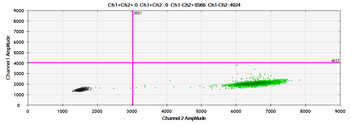


Fig.1- Representative example of 2D graph obtained by analyzing *KIT* D816V mutation on healthy donors (HDs).

- In HMC 1.2 cell dilutions, KIT D816V mutation could be detected down to 0.01% allele burden (Figure 2)

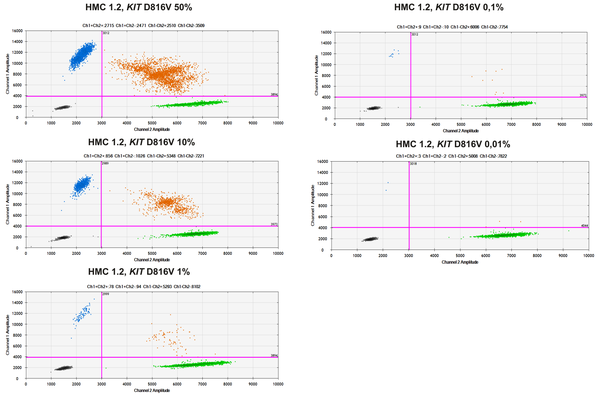


Fig.2- 2D graphs obtained with serial dilutions of KIT D816V-mutated (HMC-1.2) and unmutated (HMC-1.1) cell lines mimicking different allele burdens, from 50% to 0,,01%

- Comparison of ddPCR and ASO-qPCR revealed very high concordance in mutation detection and quantitation
- ddPCR revealed high concordance in mutation quantitation between BM and PB, even at very low variant allele frequencies (Figure 3)

Validation cohort
60 SM patients:
20 WT for *KIT* D816V on BM
40 MUT for *KIT* D816V on BM

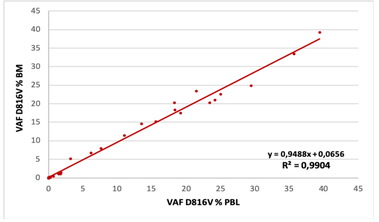


Fig.3- Scatter plot showing linear regression and correlation between *KIT* D816V mutation detected in BM and in PB

Conclusions

- ➢ ddPCR is an attractive alternative to ASO-qPCR for KIT D816V mutation detection and quantification in SM
- ➢ ddPCR may reliably detect the D816V mutation non-invasively in PB in patients with suspected SM

I have nothing to disclose