A Novel Droplet Digital PCR Strategy for Rapid and Sensitive Detection of BCR::ABL1 Kinase Domain Mutations Conferring Resistance to Second-Generation Tyrosine Kinase Inhibitors

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Background

Testing for BCR::ABL1 kinase domain (KD) mutations should always be performed before tyrosine kinase inhibitor (TKI) changes. Next-generation sequencing (NGS) is the best approach to highlight emerging mutations in patients not responding adequately to TKI therapy. However, NGS requires sample centralization and batch analysis and has a non-negligible time to results. In this study, we set up and validated a novel droplet digital PCR (ddPCR)-based multiplex strategy for the detection and quantitation of transcripts harboring mutations impacting on TKI selection.

Materials & Methods

In collaboration with Bio-Rad, a 3-tube ddPCR strategy was designed that enables identification and quantitation of 16 nucleotide substitutions encoding the 13 mutations associated with resistance to one or more second-generation TKI (2GTKI)(Table 1).Primers and FAM- or FAM/HEX-labelled probes were grouped on a TKI-specific basis and generated clusters of droplets mapping to spatially distinct areas of the 2D plot based on resistance profiles (Figure 1). Each tube also incorporated primers and HEX-labelled probes for e13a2, e14a2, and e1a2 BCR::ABL1 fusion transcripts to express results as the percentage of mutation-positive over total BCR::ABL1 transcripts. For validation, a total of 101 RNA samples from healthy donors, TKI-sensitive and -resistant patients, and BCR::ABL1-positive and -negative cell lines were used. cDNA (125 ng) obtained with ABL1-specific primers was analyzed in duplicate on a QX200 ddPCR system (Bio-Rad).

TUBES ddPCR	Amino acids substitution	Nucleotide substitution
TUBE 1	T315I	1137 C > T
TUBE 2	F317L	1142 T > C
	F317L	1144 C > A
	F317L	1144 C > G
	F317I	1142 T > A
	F317C	1143 T > G
	F317V	1142 T > G
	V299L	1088 G > C
	V299L	1088 G > T
	T315A	1136 A > G
TUBE 3	Y253H	950 T > C
	E255V	957 A > T
	E255K	956 G > A
	F359V	1268 T > G
	F359I	1268 T > A
	F359C	1269 T > G

Table 1– Amino acid and nucleotide substitutions detected by assays. Nucleotide coordinates according to the NM_005157.6 reference sequence.

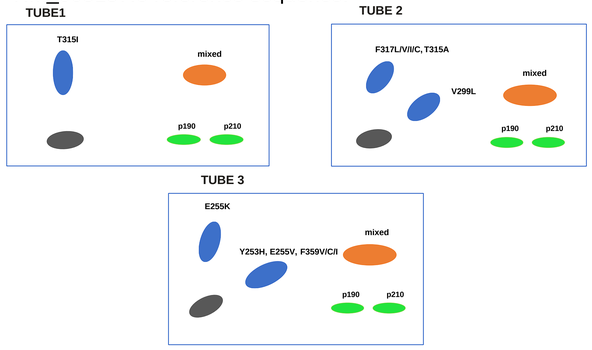


Figure 1– Schematic representation of the expected output for tube 1, 2 and 3

Results

The limit of blank was determined using 60 blank samples. Accuracy and specificity were confirmed using 48 samples positive for one or more 2GTKI-resistant mutations or mutations at nearby codons (to exclude cross-reactivity). (Figure 2)

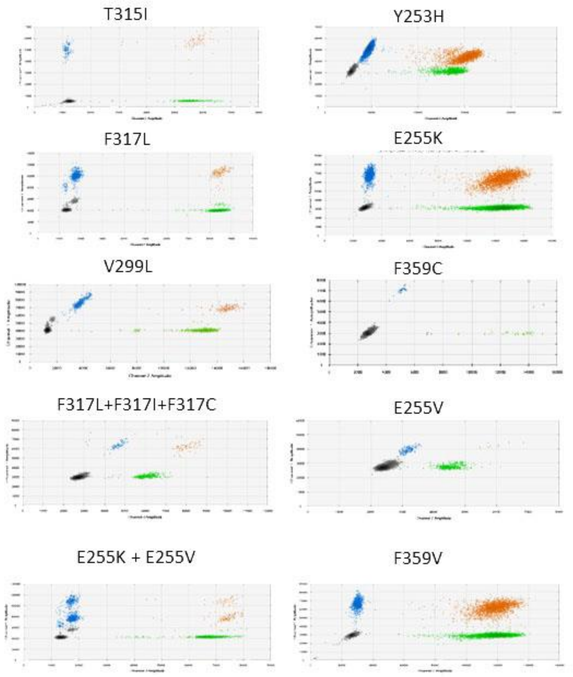


Figure 2.- Representative examples of 2D plots obtained with samples positive for 2GTKI resistant mutations

Analysis of serial dilutions of cell line mixtures made using BCR::ABL1-positive mutation-positive cells, BCR::ABL1-positive unmutated cells, and BCR::ABL1-negative cells to mimic different mutation frequencies (70%, 5%, and 0.5%) and different transcript levels (MR0 to MR3) showed that a 0.5% lower detection limit could be consistently achieved irrespective of BCR::ABL1 levels (Figure 3).

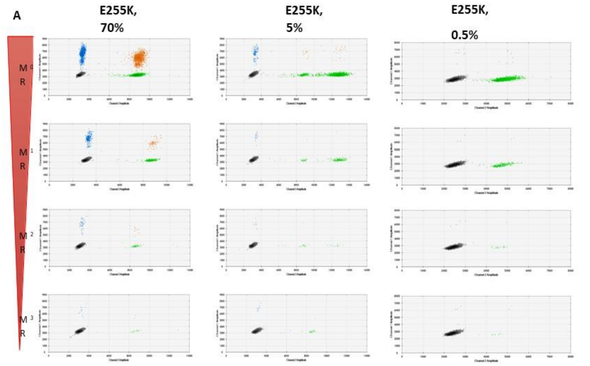


Figure 3 – Results obtained with serial dilutions of mutated into unmutated BCR::ABL1 cell lines and BCR::ABL1-negative cell lines mimicking different BCR::ABL1/ABL1 transcript levels

Conclusions

ddPCR proved highly sensitive and accurate. Total hands-on time was approximately 2 hrs, and time from sample to results was 2 days. Therefore, ddPCR may be integrated into diagnostic algorithms of CML (and Ph+ ALL) patients as a convenient first-level screening tool for mutations impacting on TKI selection.

References

Simona Soverini et al. DROPLET DIGITAL PCR FOR THE DETECTION OF SECOND-GENERATION TYROSINE KINASE INHIBITOR RESISTANT BCR::ABL1 KINASE DOMAIN MUTATIONS IN CHRONIC MYELOID LEUKEMIA. Leukemia. 2022;36(9):2250-2260. doi:10.1038/s41375-022-01660-8