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634.MYELOPROLIFERATIVE SYNDROMES: CLINICAL AND EPIDEMIOLOGICAL

Performance and Interlaboratory Reproducibility of Droplet Digital Polymerase Chain Reaction (ddPCR) and Real Time PCR for KIT D816V Mutation Detection: A Nationwide Pilot Study By the RIMA (Rete Italiana Mastocitosi) Association

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Systemic Mastocytosis (SM) is a rare hematological neoplasm, but its incidence is probably underestimated because of the heterogeneity of clinical symptoms and presentation that may cause diagnostic difficulties especially outside reference centers. Detection of the activating D816V KIT mutation in the bone marrow or peripheral blood is one of the minor criteria for the diagnosis of SM, but it poses some challenges since in the indolent forms (the most frequent) the mast cell burden is very low. For this reason, the National Comprehensive Cancer Network and the European Competence Network on Mastocytosis (ECNM) recommend a high-sensitivity PCR-based assay, such as Allele-Specific Oligonucleotide-Real Time Quantitative PCR (ASO-qPCR) or ddPCR, while Sanger and Next Generation Sequencing are considered not adequate. Accurate quantitation of allele burden (AB) is also desirable since it offers diagnostic (an AB³10% qualifies as a B-finding according to the latest WHO classification) and prognostic information and might be used to monitor response to tyrosine kinase inhibitors.

Despite the widespread routine availability of real time and digital PCR technologies, a map of labs across Italy offering KIT D816V testing with adequate sensitivity is lacking, nor have cooperative efforts aimed to check lab performance and reproducibility of diagnostic results ever been performed. Based on these premises, RIMA undertook, with the sponsorship of the GIMEMA Working Party on Chronic Myeloproliferative Neoplasms, a project aimed to: i) build a nationwide network of competent reference laboratories performing KIT D816V mutation testing; ii) promote harmonization of local procedures and ensure adequate proficiency and cross-comparability of results.

The pilot phase of this project involved 7 labs (L) spread across the country (Milan, Bologna, Verona, Florence, Rome, Naples, Pavia) serving one or more reference clinical centers for the diagnosis and management of SM. First, a survey aimed to assess the techniques and procedures in use in each participating lab was conducted. Five labs (L2-L6) used a commercial ddPCR assay (KIT p.D816V c.2447A>T, Assay ID dHsaCP2000023; Bio-Rad); one lab (L7) used a home brew ASO-qPCR assay; one lab (L1) used a commercial semi-quantitative real time PCR-based assay (PlentiPlex Mastocytosis D816V kit; Pentabase). All labs performed analyses in duplicate or triplicate using DNA as input material, with amounts variable from 50 to 250ng/replicate. Reported limit of detection (LoD) was 0.0003% for the home brew method and 0.01% for the commercial methods. Subsequently, a round robin test to evaluate and compare performance in terms of sensitivity and inter-lab reproducibility was carried out. Mutated and wild-type DNA isolated from HMC-1.2 and HL-60 cells, respectively, was mixed and diluted to mimic different D816V ABs, from 10% down to 0.01%. Dilutions were externally assessed by the UK Wessex Genomics Laboratory Service, using an accredited ddPCR assay with a limit of detection (LoD) of 0.01%. Of note, the sixth dilution (D6) was scored as slightly below their LoD (0.008%; 3 positive droplets only). Identical batches of blinded vials were then distributed and analyzed in parallel by the 7 participating labs according to their own routine protocols. Positivity/negativity as scored by L1-7 and AB values as scored by L2-7 are detailed in Table 1. All the evaluated methods proved highly accurate in the detection and, for ddPCR and ASO-qPCR, in the quantitation of the KIT D816V (R^2 between 0.988 and 0.998). A very high degree of concordance was achieved, for all dilutions, across different labs and methods (CV between 0.07 and 0.8). D6 was scored borderline by L1 and positive by all other labs.

This pilot experience demonstrates that the evaluated PCR-based methods may reliably identify and quantitate the KIT D816V mutation down to an AB of 0.01% with a high degree of cross-comparability of results, thus assisting clinicians in the diagnosis and monitoring of SM patients. To the best of our knowledge, this is the first cooperative effort aimed to assess the performance and reproducibility of KIT D816V mutation testing in SM. Involvement of additional Italian labs is already planned, and further implementation within the ECNM will be proposed. Definition of common SOPs, uniform sample requirements and web-based reporting following the GIMEMA LabNet model will be pursued.

Disclosures Guglielmelli: *GSK:* Speakers Bureau; *Abbvie:* Other: Other member of advisory board, speaker at meeting, Speakers Bureau; *Novartis:* Other: Other member of advisory board, speaker at meeting, Speakers Bureau. **Papayannidis:** *Pfizer, Astellas, Janssen, GSK, Blueprint, Jazz Pharmaceuticals, Abbvie, Novartis, Delbert Laboratoires:* Membership on an entity's Board of Directors or advisory committees; *Abbvie, Astellas, Servier, Menarini/Stemline, BMS, Pfizer, Amgen, Janssen, Incyte, Novartis:* Honoraria. **Bonifacio:** *Clinigen:* Membership on an entity's Board of Directors or advisory committees; *Incyte:* Membership on an entity's Board of Directors or advisory committees; *Novartis:* Membership on an entity's Board of Directors or advisory committees; *Pfizer:* Membership on an entity's Board of Directors or advisory committees; *BMS:* Membership on an entity's Board of Directors or advisory committees. **Pagano:** *Moderna:* Honoraria; *AstraZeneca:* Honoraria; *Novartis:* Honoraria; *Gilead:* Honoraria; *Janseen:* Honoraria; *Menarini:* Honoraria; *Pfizer:* Honoraria; *Jazz:* Honoraria. **Vannucchi:** *GSK:* Honoraria; *Abbvie:* Honoraria; *Roche:* Honoraria; *AOP:* Honoraria; *BMS:* Honoraria; *Novartis:* Honoraria; *Incyte:* Honoraria. **Cavo:** *Sanofi:* Consultancy, Honoraria; *Celgene/Bristol Myers Squibb:* Consultancy, Honoraria, Speakers Bureau; *GlaxoSmithKline:* Honoraria; *Roche:* Honoraria; *AbbVie:* Consultancy, Honoraria; *Amgen:* Honoraria; *Janssen:* Consultancy, Honoraria, Speakers Bureau; *Adaptive:* Honoraria; *Takeda:* Honoraria.

TABLE 1

DILUTIONS	Expected AB	WGLS	L1	L2	L3	L4	L5	L6	L7	MEDIA	SD	CV
D1	10%	9.39	Pos	11.60	12.26	12.30	11.76	12.19	7.14	11.208	1.838	0.164
D2	1%	1.27	Pos	1.64	1.67	1.70	1.47	1.64	1.42	1.590	0.106	0.066
D3	0.50%	0.73	Pos	0.95	0.81	1.01	0.88	0.90	0.82	0.895	0.070	0.078
D4	0.10%	0.11	Pos	0.15	0.15	0.17	0.17	0.13	0.12	0.148	0.019	0.126
D5	0.05%	0.04	Pos	0.08	0.07	0.09	0.08	0.07	0.04	0.072	0.016	0.219
D6	0.01%	0.008	Borderline	0.04	0.01	0.04	0.01	0.01	0.004	0.019	0.015	0.789

KIT D816V mutation burden and summary statistics (media, standard deviation [SD], coefficient of variation [CV]) of results obtained by the Wessex Genomics Laboratory Service (WGLS) and the seven participating laboratories.

Figure 1

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