Molecular and Cellular Probes 77 (2024) 101976

Contents lists available at ScienceDirect

Molecular and Cellular Probes

journal homepage: www.elsevier.com/locate/ymcpr

Performance of the Idylla microsatellite instability test in endometrial cancer

Marta Mendiola [a,b,*,4], Victoria Heredia-Soto [b,c,4], Ignacio Ruz-Caracuel [b,d,1], Amparo Baillo [e], Jorge Luis Ramon-Patino [f,3], Alberto Berjon [a,d], Francisco Javier Escudero [c,2], Alberto Pelaez-Garcia [a], Alicia Hernandez [g,h], Jaime Feliu [b,c,f,h,i], David Hardisson [a,b,d,h], Andres Redondo [c,f,h,i,**]

[a] Molecular Pathology and Therapeutic Targets Group, Hospital La Paz Institute for Health Research (IdiPAZ), 28046, Madrid, Spain
[b] Center for Biomedical Research in the Cancer Network (CIBERONC), Instituto de Salud Carlos III, 28029, Madrid, Spain
[c] Translational Oncology Research Laboratory, Hospital La Paz Institute for Health Research (IdiPAZ), 28046, Madrid, Spain
[d] Department of Pathology, La Paz University Hospital, 28046, Madrid, Spain
[e] Mathematics Department, Autonomous University of Madrid, 28049, Madrid, Spain
[f] Department of Medical Oncology, La Paz University Hospital, 28046, Madrid, Spain
[g] Department of Obstetrics and Gynecology, La Paz University Hospital, 28046, Madrid, Spain
[h] School of Medicine, Autonomous University of Madrid, 28046, Madrid, Spain
[i] Cátedra UAM-ANGEM, Faculty of Medicine, Autonomous University of Madrid, 28046, Madrid, Spain

ARTICLE INFO

Keywords:
Endometrial carcinoma
Mismatch repair deficiency
Microsatellite instability
Idylla

ABSTRACT

Context: DNA mismatch repair (MMR) deficiency (dMMR) testing is now recommended in endometrial cancer. Defect identification in the molecules participating in this pathway, or the presence of microsatellite instability, are commonly employed for this purpose. Novel methods are continuously evolving to report dMMR/microsatellite instability and to easily perform routine diagnoses.

Objective: The main aim of this study was to compare the concordance of the Idylla microsatellite instability test for the identification of dMMR endometrial cancer samples defined by immunohistochemistry and MMR genomic status.

Design: We applied the Idylla MSI test to 126 early-stage endometrial cancer cases with MMR testing by immunohistochemistry and genomic characterization (methylation in *MLH1* and sequence alterations in *MLH1*, *PMS2*, *MSH2* and *MSH6*). Individual markers and overall specific performance indicators were explored.

Results: The Idylla platform achieved a higher global concordance rate with MMR genomic status than with immunohistochemistry (75 % and 66 %, respectively). Sensitivity and specificity are also higher (75 % vs 66 % and 96 % vs 90 %, respectively). Clustering analysis split the patients into 2 well-differentiated clusters, the pMMR and the dMMR group, represented by MLH1/PMS2 loss and the *MLH1* methylated promoter. Overall, immunohistochemistry and MMR genomic status identified more dMMR cases than did the Idylla test, although correlations were improved with a modified Idylla test cut-off.

Conclusions: Performance of the Idylla test was better correlated with MMR genomic status than MMR immunohistochemistry status, which improved with a modified test cut-off. Further studies are needed to confirm the cut-off accuracy.

* Corresponding author. Molecular Pathology and Therapeutic Targets Group, La Paz University Hospital (IdiPAZ), Paseo de la Castellana, 261, 28046, Madrid, Spain.

** Corresponding author. Department of Medical Oncology, Hospital Universitario La Paz, IdiPAZ, Faculty of Medicine, Universidad Autónoma de Madrid, Paseo de la Castellana, 261, 28046, Madrid, Spain.

E-mail addresses: marta.mendiola@salud.madrid.org (M. Mendiola), andres.redondos@uam.es (A. Redondo).

[1] Current address: Department of Pathology, Hospital Ramón y Cajal, IRYCIS, Madrid, Spain.
[2] Current address: Area of Human Genetics, Institute for Rare Diseases Research (IIER), Instituto de Salud Carlos III, Madrid, Spain.
[3] Current address: START, CIOCC (Centro Integral Oncológico Clara Campal), Madrid, Spain.
[4] Contributed equally to this work.

https://doi.org/10.1016/j.mcp.2024.101976
Received 24 June 2024; Received in revised form 25 July 2024; Accepted 25 July 2024
Available online 31 July 2024

1. Introduction

Mismatch repair (MMR) is one of the main DNA repair mechanisms. Inactivation of its essential components (MLH1, PMS2, MSH2, and MSH6) causes MMR pathway deficiency (dMMR) [1,2]. This phenotype is present in 20%–30 % of endometrial carcinoma (EC) cases. The majority of dMMR tumors are sporadic, with *MLH1* promoter methylation the most frequently found defect [3]. Alterations in the *MLH1*, *PMS2*, *MSH2*, and *MSH6* sequences can also be the cause of the MMR deficiency, which can be somatic or germline acquired (the latter is linked with a predisposition to Lynch syndrome). MMR status determination is now recommended in EC guidelines for 3 reasons: to confirm the diagnosis in cases with non-conclusive morphology; for Lynch syndrome screening; and for molecular EC subtyping, with both prognostic and therapeutic implications [4]. The comprehensive genomic study performed by The Cancer Genome Atlas (TCGA) Consortium established 4 molecular groups. The hallmark of one group is dMMR, which is associated with an intermediate risk of relapse [5]. Additionally, dMMR is now recognized as a predictive biomarker for the response to immunotherapy [6–9].

Immunohistochemistry (IHC) is the recommended first approach to dMMR testing in EC clinical practice [10,11]. A sample is considered proficient MMR (pMMR) when the expression of all markers is preserved, whereas the loss of expression in one or more markers indicates deficient dMMR [12]. IHC is routinely used in pathology departments; however, there are other methods to identify the gene alterations that cause the dMMR phenotype. As an alternative approach, targeted next generation sequencing (NGS) panels can be used to detect sequence alterations in MMR genes, and methylation-specific multiplex ligation-dependent probes (MS-MLPA) or bisulfite conversion DNA-based methods can be employed for finding *MLH1* promoter methylation events [5,13–15]. Microsatellites are short repeated sequences distributed along the DNA that are prone to errors due to their structure. Variations in their length can occur, and when MMR is not working properly these mistakes are not efficiently repaired. This is referred to as microsatellite instability (MSI), linked to MMR defects, and can be used as a dMMR surrogate [16,17]. MSI can be detected mainly by the polymerase chain reaction (PCR) amplification of microsatellite regions and Sanger capillary electrophoresis due to their size discrimination [18].

Two panels widely used for decades have been the Bethesda, which involves the analysis of 2 mononucleotide (BAT-25 and BAT-26) and 3 dinucleotide (D5S346, D2S123 and D17S250) microsatellites, and the Promega MSI Analysis version 1.2 kit, including 5 mononucleotide microsatellites (BAT-25, BAT-26, NR-21, NR-24, NR-27) [19,20]. Additional PCR-Sanger-based panels, as well as others based on PCR and high resolution melting (HRM) or microfluidic electrophoresis, are now available as alternative options [21]. NGS panels have also been developed, although the advanced technical requirements and the variability in the microsatellites explored make them difficult to standardize and routinely implement [22,23]. Each method evaluates different microsatellites, and few comparative studies are available to fully evaluate their performance. All these approaches compare the number of repeats between normal and tumor tissue from the same individual, or establish thresholds for these repeats in the tumor sample to set the MSI status. Regarding PCR-based methods, MSI status is divided into MSI-high (MSI-H), when 2 or more microsatellites are unstable, or MSS, when only 1 microsatellite is unstable or no instability is observed [10]. The Idylla MSI Test explores 7 biomarkers (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, and SULF2) that are frequently altered in MSI cancers, analyzes their size, and establishes their instability by a previously fixed threshold in a fully automated way by HRM [24].

The aim of this study was to evaluate the performance of the Idylla MSI test in a series of early-stage EC cases in which the MMR/MSI status had previously been defined by IHC and MMR genomic testing.

2. Materials and methods

2.1. Patients and samples

This study was approved by the local Ethics Committee (HULP #PI3778) and performed in a previously explored early-stage EC series from la Paz University Hospital [25]. An initial selection of 289 patients was included, based on EC diagnosis between 2003 and 2015 and tissue availability.

IHC and MMR genomic status were previously performed [26]. For IHC, the expression of MLH1, PMS2, MSH2, and MSH6 was evaluated in tissue microarray (TMA) sections. dMMR was assigned when the complete loss of nuclear expression of 1 or more markers was observed in tumor cells [12]. The final study population included 126 cases distributed in 2 groups: the first consisted of the 67 (53.2 %) dMMR tumors, and the second consisted of 59 (46.8 %) tumors randomly selected among the pMMR population [26]. DNA from the selected cases was employed for genomic status evaluation. *MLH1*, *PMS2*, *MSH2*, and *MSH6* were explored with a custom panel (Paragon Genomics, Hayward, CA, USA) and sequenced in MiSeq (Illumina, San Diego, CA, USA). Pathogenic mutations were reported employing VarSome and Integrative Genomics Viewer (IGV) software [27,28]. The *MLH1* promoter was also explored by an MS-MLPA ME011 kit (MRC Holland, Amsterdam, Netherlands) to identify CpG methylated islands [26].

2.2. Idylla test

The Idylla MSI assay was performed in their proprietary system, directly loading into the cartridge DNA extracted from tissue block punches of selected areas containing as much tumor as possible, with a minimum content of 40 % of tumor cells [29–31]. This test is automated for the entire process, including MSI status reporting. The 7 microsatellite targets are amplified by specific primers, detected by fluorescently-labeled molecular beacons and separated by size with HRM. The proprietary software first checks the validity of each marker, and MSI status is determined when a valid result is obtained in at least 5 of the 7 markers. Second, it performs pattern recognition to assign a probability score of whether it has been altered. Each biomarker is analyzed individually, with values greater than 0.5 considered associated with an unstable pattern. MSI-H is determined when at least 2 markers are unstable, and otherwise it is MSS.

2.3. Statistical analysis

Contingency tables for the IHC markers, the NGS status, and the Idylla MSS/MSI-H classes were computed for all pairs of categorical variables of interest. The results of the 3 screening methods were used to perform Fisher's exact test of independence to check for associations between the variables. Phi (correlation) coefficients were computed for all pairs of dichotomous variables to describe and detect pairwise dependence between them. Furthermore, logistic regressions were fitted to study the capability of groups of markers to classify into two groups (determined in each specific regression context by a certain dichotomous outcome of one of the screening procedures), e.g., to decide which IHC markers were significant in the logistic discrimination between the 2 possible NGS results. The diagnostic performance of these regressions was quantified by the overall proportion of correct classifications as well as the sensitivity and the specificity. A p-value of 0.05 or less was considered significant. For the quantitative microsatellite probability scores, the (Pearson) correlation matrix was computed and a principal components analysis was performed. Assigning a patient whose microsatellite sum is above a certain threshold to the MSI status has been shown to improve, in general terms, the classification into IHC and genomic status. Finally, a k-prototype cluster analysis was simultaneously performed on all the IHC markers, the NGS status, and the Idylla microsatellite values, yielding a division of the patients into

homogeneous groups, each group corresponding to a patient subtype. These representatives highlight the main agreements among the various markers. Missing data for the specific variables under consideration in a statistical procedure were removed in the statistical analysis (only for that procedure). All the inferential procedures were performed with base R and the k-prototype method with the clustMixType R library.

3. Results

3.1. Descriptives

Of the 126 tumors in the total study sample, 2 (1.6 %) cases were not validated with the Idylla test due to failures in the MS amplification process and were excluded for further comparisons. Among the remaining 124 cases, 98 (79.1 %) were classified as MSS and 26 (20.9 %) as MSI-H. The distribution of MS alteration patterns in the latter group is summarized in Fig. 1, observing that the most frequent unstable MS was DIDO1 and the least RYR3. One sample has been removed due to a missing SULF2 value.

3.2. Comparison between Idylla microsatellite instability test and immunohistochemistry

A crosstab containing the distribution of cases by both categories is given in Table 1.

Regarding categories for each technique, the overall concordance was high for the pMMR and MSS categories, with 54 pMMR cases considered as MSS, and 3 cases assigned as MSI by Idylla (94.7 % and 5.5 % among the pMMR population, respectively). However, major discrepancies were observed in the dMMR group: whereas 23 cases were considered MSI-H by Idylla, another 44 dMMR cases were classified as MSS (34.3 % and 65.7 % among the dMMR population, respectively).

Correlation between IHC and Idylla was 0.35, with a coincidence proportion of 62.1 %. Fisher's exact test exploring the MSI categories versus the IHC results showed a p-value of 5.08e-05, demonstrating dependency between results of both techniques.

As a secondary analysis, we explored the IHC results by individual markers and tandems, summarized in Supplementary Table 1.

The proportion of correctly classified cases was higher for MLH1 and MSH2 (75 %), followed by PMS2 (72.6 %) and MSH6 (67.7 %). Regarding IHC doublets, MSH2/MSH6 showed more correctly classified cases than MLH1/PMS2 (72.6 % and 67.7 %, respectively). Fisher's exact test provided significant p-values only for MLH1, PMS2, and their tandem (7.43e-05, 1.32e0-3, and 5.87e-4 respectively), demonstrating dependency between variables.

Table 1
Two-way contingency table for MSI Idylla status and a) MMR IHC and b) Genomic status groups.

		a) IHC		b) Genomic status		
		dMMR	pMMR	Altered	No	Total
Idylla test	**MSS**	44	54	30	68	98
	MSI	23	3	25	1	26
	Total	67	57	55	69	124

MSS: microsatellite stable; MSI: microsatellite instability; IHC: immunohistochemistry; MMR: mismatch repair, dMMR: deficient MMR; pMMR: proficient MMR.

3.3. Comparison between Idylla microsatellite instability test and mismatch repair genomic status

A crosstab containing distribution of cases by both categories is included in Table 1.

Regarding categories for each technique, the overall concordance was high for the group with no genomic alterations and MSS, with 68 cases without genomic alterations assigned as MSS by the Idylla test, and 1 case reported as MSI (98.5 % and 1.5 % within the dMMR population, respectively). As occurred with IHC, major discrepancies were observed in the other category: whereas 25 cases with genomic alterations were assigned to the MSI category, another 30 cases were considered as MSS by the Idylla test (45.5 % and 54.5 % within the dMMR population, respectively).

The correlation between genomic status and the Idylla test was 0.54, and the overall coincidence was 75.0 %. Fisher's exact test exploring the 3 MSI categories versus the IHC result showed a p-value of 5e-10, demonstrating dependency between results of both techniques.

We explored as a secondary analysis the genomic status results by individual markers, and the results are summarized in Supplementary

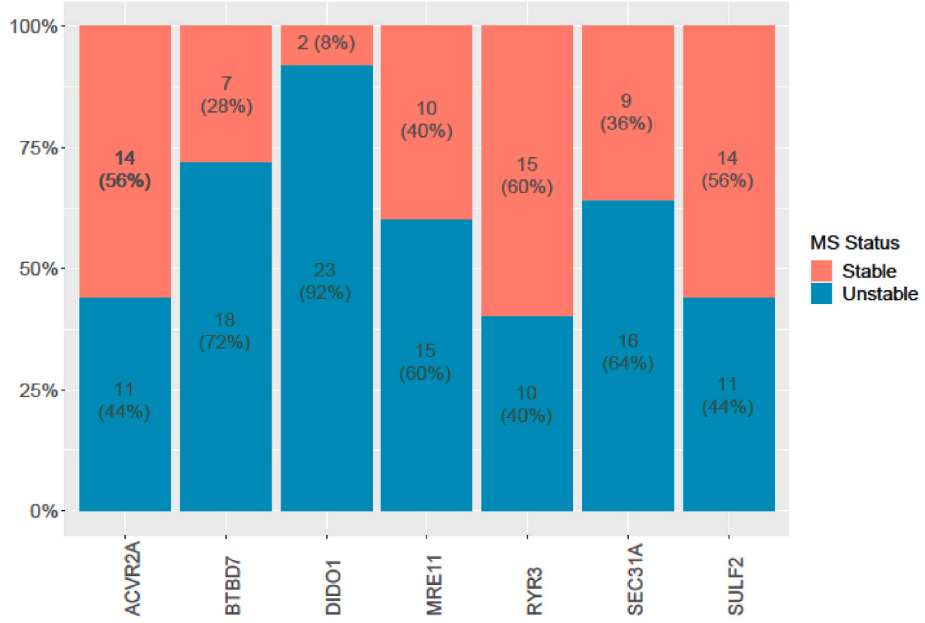

Fig. 1. Distribution of instability among Idylla markers in the EC MSI-H population. Markers are distributed by columns and y axis indicate percentage. Stable (red) and unstable (blue) number of cases and percentages for each marker are indicated.

Table 2.

The exploration gene by gene showed that the proportion of correctly classified cases was higher for *MLH1*, followed by *MSH2* and *PMS2*, and lastly *MSH6*. Marker by marker, IHC and PCR displayed a lower degree of mutual dependence and ability to predict each other than the Idylla test and MMR gene status.

Regarding *MLH1*, the promoter methylation and coding sequence alterations were explored separately. The proportion of concordant classified cases was higher for the *MLH1* promoter methylation category than for the mutations in its sequence (87.1 % and 75.6 %, respectively). Fisher's exact test provided significant p-values only for methylated *MLH1* and *MLH1* overall status (2e-11 and 7e-10, respectively). None of the sequencing status by gene showed significant dependencies.

3.4. Overall concordance comparisons

A complete summary of the sensitivity, specificity, and overall concordances is shown in Table 2. Taking IHC as the reference, the proportion of correct Idylla test predictions is lower than those for MMR genomic status (62.1 % vs 75.8 %, respectively), showing similar sensitivities (88.5 % and 83.6 %, respectively) and low specificity (55.1 % vs 69.6 %, respectively). Taking the MMR gene status as reference, the proportion of correctly classified cases was similar for Idylla and IHC (75.0 % and 75.8 %, respectively), although a higher sensitivity and a lower specificity was observed for the Idylla test (96.2 % vs 68.7 % and 69.4 % vs 84.2 %, respectively).

Overall, the IHC and Idylla displayed a lower degree of mutual dependence and ability to predict each other than the Idylla test and MMR gene status. This situation was also reflected in the correlations and concordance proportions separated by markers, as previously stated. In summary, there was acceptable concordance between the MSS and pMMR categories but a higher proportion of discrepancies between MSI and dMMR, when comparing Idylla results and IHC or MMR gene status.

3.5. Clustering exploration

With the aim of exploring the simultaneous concordance between all the markers and detecting homogeneous groups among the observed patients, we applied a clustering procedure to the following variables: Idylla MS scores, IHC, and MMR genomic status markers. Given that the variables were of mixed type (quantitative and qualitative), we employed the k-prototype clustering technique, which performs well in this context [32].

For a specific number k of clusters, k-prototypes separate the patients into k groups of similar individuals and finds a representative (the prototype) in each of them. The simplest procedure to select the optimal number of clusters is a scree plot that plots, for each k, the total within sums of distances to the prototypes. This procedure identifies $k = 2$ as a satisfactory number of clusters that provides an adequate separation of patients (Fig. 2a). As a result, we can see that there is acceptable concordance between the 3 dMMR methods under consideration. We performed the 2-prototypes procedure, obtaining the classification of the patients into either of the 2 clusters, and the marker values of the prototypes (Fig. 2b), The differential features of the 2 representatives are very clear. For prototype 1, the values of all the Idylla MSs are below 0.03 (thus, stable MSs), all the IHC markers are preserved, and none of the genomic markers are altered, indicating that this subtype reflected the pMMR profile. In the case of prototype 2, all the Idylla MSs are above 0.45 (thus, unstable or close); the IHC tandem MLH1/PMS2 is lost, but the tandem MSH2/MSH6 is present; regarding MMR genomic status, only *MLH1* was altered by promoter methylation. These characteristics show that subtype 2 reflected the most common dMMR profile. By assigning a patient to the nearest prototype, 94 patients were classified into cluster 1 and the remaining 26 into cluster 2.

Table 2
Global comparisons between tests. IHC and Genomic status are set as reference.

Reference	Compared variable	Sensitivity	Specificity	Proportion of correctly classified cases
IHC	**Idylla test**	88.5	55.1	62.1
	MMR genomic status	83.6	69.6	75.8
MMR genomic status	**Idylla test**	96.2	69.4	75.0
	IHC	68.7	84.2	75.8

MMR, mismatch repair; IHC: immunohistochemistry.

3.6. Proposed alternative scoring Idylla test model

The computed correlation matrix between the MS status of the Idylla test appears in Fig. 3a. Note that all correlation indices are positive and greater than 0.55, and the determinant of the correlation matrix is 0.01; thus, 1 or possibly more MSs are redundant in the sense that they can be explained by a lineal combination of the others.

We have computed the principal components (PCs) of the observed MS values. Fig. 3b shows the proportion of total variance explained by each of the components. As expected from the high correlations between the MSs, the bar plot shows that the first PC explains a high proportion of the total variability (73.0 %). Thus, we are able to summarize almost 3/4 of the information of the 7 MSs with just 1 variable (the first PC). Fig. 3c plots the weight of each MS in the first component, observing that the coefficients are similar in magnitude. Therefore, the first PC is a type of weighted average of all the Idylla MSs and each MS has approximately the same importance in the PC, which (see below) will be employed for the assignation of MSI status. Instead of using the values of the 7 MSs separately to set the MSI status, it makes sense to examine only the value of the first PC and establish a threshold for the component above. This approach has the advantage of making the most of the information gained from the MS numerical values. For instance, according to the usual procedures, a patient with all MS values below 0.5 would be assigned an MSS status even if all the MS values were near 0.5.

Keeping in mind that discrepancies are greater in dMMR cases judged as MSS by the Idylla test, it can be hypothesized that decreases in the established Idylla cut-off value for instability (0.5), could increase the correct classification rate. To simplify the procedure, instead of taking the linear combination of the MS values given by the loadings of the first PC, we just considered the sum of all the MSs. The optimal cut-off maximizing the overall agreement when predicting the MMR genomic status was approximately 0.286; thus, the threshold (rounding to the first decimal) was fixed at 0.3. Comparisons between the specificity, sensitivity, and the global correct classification rates with the 0.5 individual threshold and the 0.3 cut-off for the sum are displayed in Table 3, where it can be seen that all but one of the results improve with the new procedure both in IHC and MMR genomic status.

4. Discussion

The aim of this study was to compare the Idylla MSI test with IHC and MMR genomic status for the identification of dMMR/MSI EC cases, with the aim of evaluating their equivalence. Initial screening was performed by IHC, selecting a population with approximately half dMMR and pMMR cases (53.17 and 46.83 %, respectively). Of these, 43.65 % of cases presented genomic alteration and 54.76 % did not. IHC is currently considered the first-choice technique for dMMR evaluation, based on the expression of 4 markers: MLH1, PMS2, MSH2, and MSH6. However, some protein defects that could interfere with their functionality can still retain their expression, resulting in a false negative interpretation [33, 34]. Additionally, although DNA genomic alterations indicate a specific

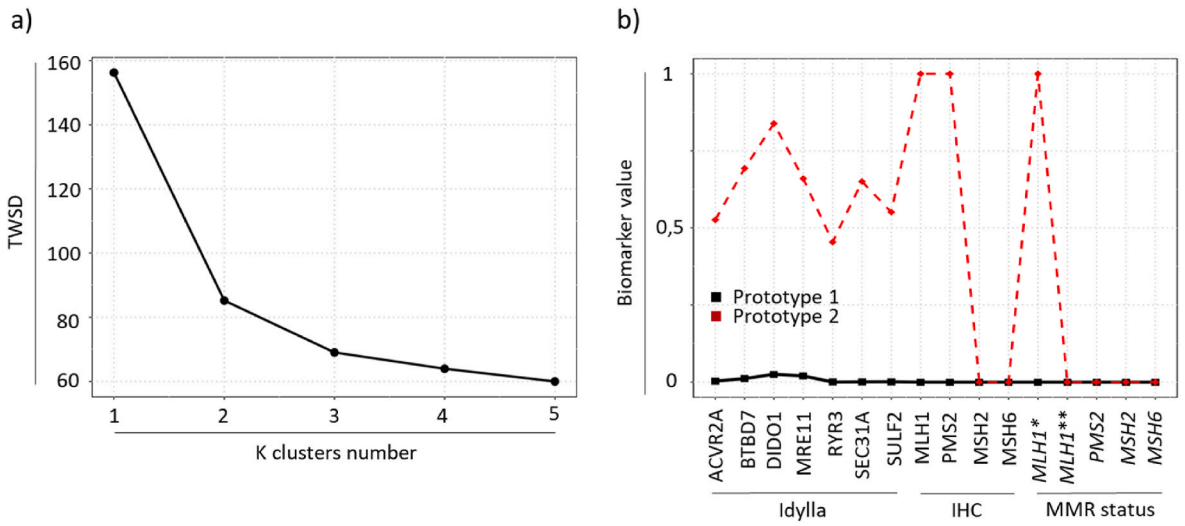

Fig. 2. Cluster analyses by k-prototypes. a) Elbow plot of Total Within Sums of Distances (TWSD) against number k of clusters; b) Prototype values corresponding to the 2 clusters representative of the patients in each group. Y axis values: "1" indicates IHC loss or MMR genes status altered, meanwhile "0" indicates preserved IHC and no alteration in MMR status. IHC: immunohistochemistry; MMR: mismatch repair; MLH1 *methylated, **mutated.

defect in a specific gene, another mechanisms as indirect alterations could occur, and account some discrepancies [35].

MSI is a direct consequence of MMR failure and supposedly explains all losses in the pathway's functionality, regardless of its cause. For this reason, this assay would theoretically identify more dMMR cases. The approaches most commonly employed for MSI determination are based on MS panels amplified by PCR and discriminated by size, employing various technologies. One of these widely used panels is composed of 5 mononucleotides (BAT-25, BAT-26, NR-21, NR-24, NR-27), and has been commercially developed [19,20].

The Idylla MSI test includes a panel of 7 tumor-specific monomorphic markers, which presents certain technical advantages: it does not need comparison to non-tumoral tissue, and it can be performed from either extracted DNA or directly from paraffin-embedded tissue sections [29,36,37]. Moreover, the system is fully automated and requires minimal handling, facilitating technical performance for clinical laboratories. Data interpretation is also minimized by the generation of a results report, which is obtained in a total time of less than 3 h. We obtained a failure rate of 1.59 %. The amount of DNA is crucial for the test, and quantification should be $>$ 2.5 ng/ul. All tested samples in the study were above this threshold; thus, the invalid results might be attributed to low quality rather than quantity. However, our invalid results are in the range previously reported in other studies, below 5 % [38,39].

The Idylla test has been evaluated previously in EC. Although the specificity appears to be very high, approximately 100 %, more variability in sensitivity has been reported among studies, ranging from 58 % to 100 %. These studies are difficult to compare, given that the study population distributions are different; overall, however, discrepancies were more frequently observed in the dMMR group [21,36,38–41]. Our results are in agreement with this observation, showing a high concordant classification between the pMMR and MSS categories, though with discrepancies coming to light in dMMR cases evaluated by IHC or genomic status and assigned to MSS by the Idylla test. Regarding IHC, 38 % of cases showed discrepant Idylla classification, which is higher than that reported in other studies. However, those discrepancies were more commonly associated with PMS2 and MSH6 losses, as previously stated [41]. Regarding MMR genomic status, a lower proportion but still a high number of discrepancies (25.2 %) were observed compared to the Idylla test. A study by Libera et al. reported that discrepancies included cases with methylated *MLH1*, *MSH2*, and *MSH6*. Our results are in agreement with those, showing discordances were more common in cases with *PMS2* and *MSH6* alterations [21].

IHC evaluates the final component of MMR defects: loss of protein expression. MMR gene status determines the main cause of the MMR defects, and MSI confirms a consequence of the defects that could explain some discrepancies between techniques. Additionally, it might be taking into account that most MSI testing approaches were developed for colorectal carcinoma, although it has been suggested that the MSI patterns could be specific to different tumor settings. Minimal microsatellite shifts have been reported in EC, suggesting that fine-tuning of molecular tests needs to be performed depending on the tumor [31,42]. Whether these minimal shifts have functional consequences in EC needs to be further studied. The k-prototype clustering based on the 3 explored variables suggests grouping the patients into 2 clusters, one representing the pMMR profile, and the other the dMMR cases, paired with MLH1/PMS2 loss and *MLH1* promoter methylation. This approach highlights the concordances between Idylla and the other 2 techniques in these scenarios, which are the most commonly represented.

Additionally, to achieve better concordance between techniques, some authors have suggested that the Idylla test threshold could be adapted depending on the tumor type [21,30]. In this test, each biomarker is individually scored. Taking this into account, we explored the possibility of a linear combination (e.g., a weighted mean) of all MSs, and a PC analysis of the MSs suggested that all of them should be included in the MSI score, with the same weight. Regarding the cut-off values, Gatius et al. suggested this be modified in EC from an individual 0.5 cut-off to a 0.3 threshold for the MS sum [38]. The analysis in our series supports this assumption, providing a mathematical justification for its use, with 0.3 the best cut-off to maximize the correct IHC prediction rate and MMR genomic status results.

MMR status has become a relevant biomarker, mainly as a predictor of greater immunotherapy benefit [8,9]. Although various approaches have been developed for their determination, clinical guidelines recommend IHC in EC [10,11]. Further research is needed to determine whether alternative methods could be clinically equivalent or even better in the identification of appropriate candidates for immunotherapy treatment of patients with EC.

5. Conclusions

In our study, the Idylla test was highly concordant for the pMMR assignment; however, discrepancies were observed for the dMMR cases. Better assay results were achieved when the instability degree of the MS

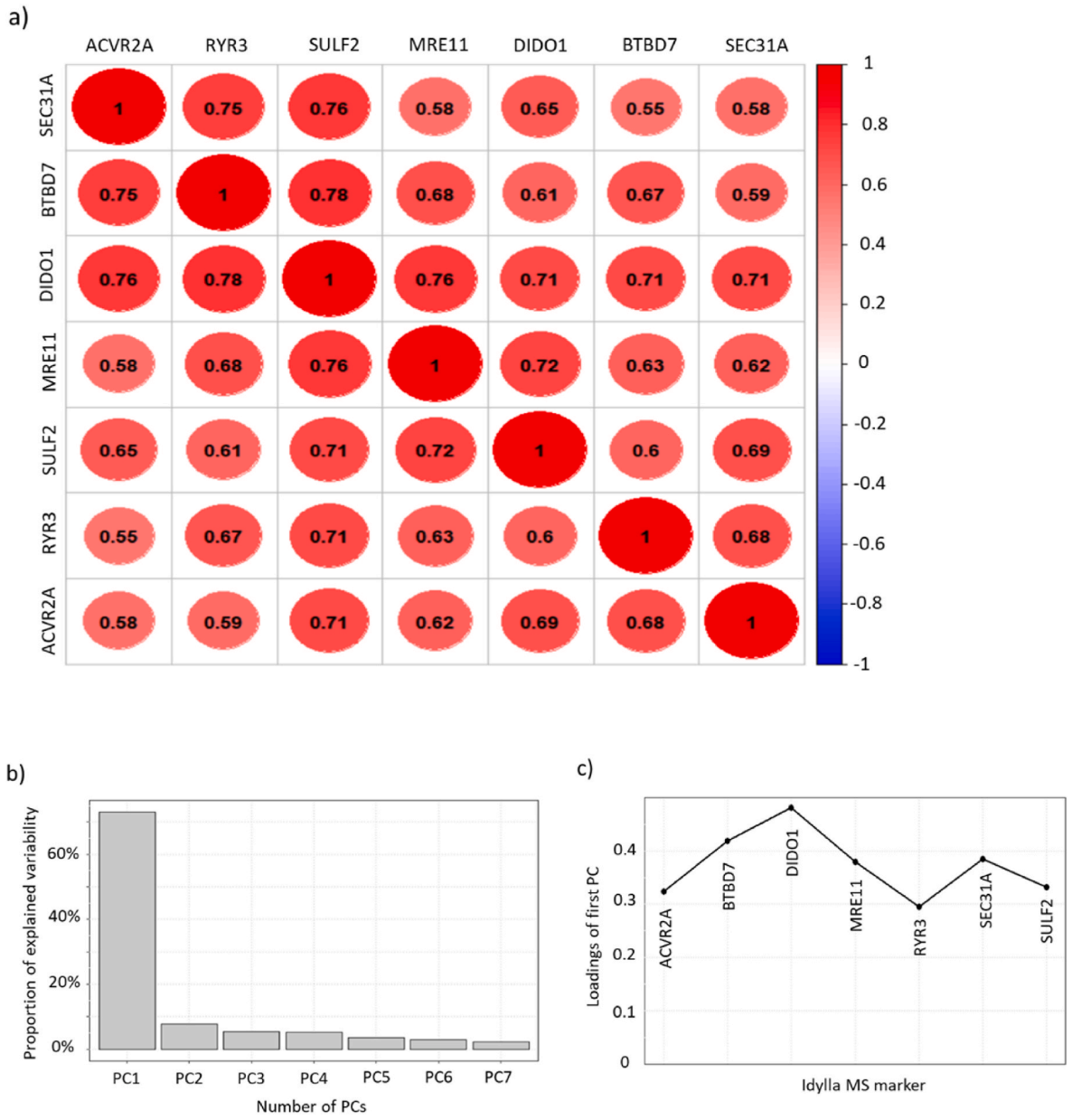

Fig. 3. a) Correlation matrix of the Idylla markers. Color scale indicates low (blue) and high values (red) and circle size higher correlation; **b)** Barplot of the variability explained by each principal component (PC) and **c)** Loadings of the first PC for the Idylla test.

Table 3
Global comparisons between test with the Idylla modified cut-off.

Reference	Compared variable: Idylla test; cut-off values	Sensitivity	Specificity	Proportion of correctly classified cases
IHC	**0.5***	88.5	55.1	62.1
	0.3**	90.6	59.3	67.5
MMR genomic status	**0.5***	96.2	69.1	74.8
	0.3**	93.8	72.2	77.9

IHC, immunohistochemistry, MMR, mismatch repair; MS, microsatellite; Cut off for * individual MS values, and ** the sum of MS values.

is summed, and the global cut-off of this scoring is reduced. Further studies are needed to validate these results and to evaluate their clinical significance, with the aim of establishing the best predictive test for immunotherapy response.

Ethics

This study was performed in compliance with relevant laws and institutional guidelines. Informed consent from participants was obtained and the privacy rights of human subjects was observed. The study was approved by the local ethics comittee and conducted in accordance with the ethical standards of the Declaration of Helsinki of the World Medical Association.

Conflict of interest

MM reports having received honoraria (MSD, AstraZeneca and GSK), research grant/funding to her institution (Eisai and PharmaMar), and

travel/accommodation/expenses (Biocartis, AstraZeneca, GSK, PharmaMar, Roche and Pfizer) outside the submitted work.

AR reports having received honoraria and providing advisory/consultancy services (MSD, AstraZeneca, Roche, GSK, Clovis, PharmaMar, Lilly, Amgen), as well as having received research grant/funding to his institution (Eisai, PharmaMar, Roche), travel/accommodation/expenses (AstraZeneca, Tesaro: A GSK Company, PharmaMar, Roche), and participating in a speaker's bureau (MSD, AstraZeneca, Roche, GSK, Clovis, PharmaMar) outside the submitted work. The remaining authors declare no conflicts of interest.

Funding

A.B. is supported by the Spanish State Research Agency (*Agencia Estatal de Investigación*) through project PID2019-109387 GB-I00. I.R.C., A.P.G., and D.H. are supported by the Instituto de Salud Carlos III (ISCIII) through grant PI21/00920, co-financed by the European Regional Development Fund 'A way to achieve Europe' (FEDER). Biocartis have supported the MSI Idylla tests.

CRediT authorship contribution statement

Marta Mendiola: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Victoria Heredia-Soto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ignacio Ruz-Caracuel:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Amparo Baillo:** Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Jorge Luis Ramon-Patino:** Writing – review & editing, Investigation, Data curation. **Alberto Berjon:** Writing – review & editing, Investigation, Formal analysis. **Francisco Javier Escudero:** Writing – review & editing, Investigation. **Alberto Pelaez-Garcia:** Writing – review & editing, Investigation. **Alicia Hernandez:** Writing – review & editing, Resources, Investigation. **Jaime Feliu:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition. **David Hardisson:** Writing – review & editing, Investigation. **Andres Redondo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank the IdiPAZ HULP Biobank and Sequencing core facilities for their support, as well as to Biocartis for goods providing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mcp.2024.101976.

References

[1] G.M. Li, Mechanisms and functions of DNA mismatch repair, Cell Res. 18 (1) (Jan 2008) 85–98, https://doi.org/10.1038/cr.2007.115.
[2] M.F. Kane, M. Loda, G.M. Gaida, et al., Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines, Cancer Res. 57 (5) (Mar 1 1997) 808–811.
[3] S.N. Shah, S.E. Hile, K.A. Eckert, Defective mismatch repair, microsatellite mutation bias, and variability in clinical cancer phenotypes, Cancer Res. 70 (2) (Jan 15 2010) 431–435, https://doi.org/10.1158/0008-5472.CAN-09-3049.
[4] N. Concin, X. Matias-Guiu, I. Vergote, et al., ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma, Int. J. Gynecol. Cancer 31 (1) (Jan 2021) 12–39, https://doi.org/10.1136/ijgc-2020-002230.
[5] N. Cancer Genome Atlas Research, C. Kandoth, N. Schultz, et al., Integrated genomic characterization of endometrial carcinoma, Nature 497 (7447) (May 2 2013) 67–73, https://doi.org/10.1038/nature12113.
[6] R.N. Eskander, M.W. Sill, L. Beffa, et al., Pembrolizumab plus chemotherapy in advanced endometrial cancer, N. Engl. J. Med. 388 (23) (Jun 8 2023) 2159–2170, https://doi.org/10.1056/NEJMoa2302312.
[7] M.R. Mirza, D.M. Chase, B.M. Slomovitz, et al., Dostarlimab for primary advanced or recurrent endometrial cancer, N. Engl. J. Med. 388 (23) (Jun 8 2023) 2145–2158, https://doi.org/10.1056/NEJMoa2216334.
[8] D.M. O'Malley, G.M. Bariani, P.A. Cassier, et al., Pembrolizumab in patients with microsatellite instability-high advanced endometrial cancer: results from the KEYNOTE-158 study, J. Clin. Oncol. : official journal of the American Society of Clinical Oncology 40 (7) (Mar 1 2022) 752–761, https://doi.org/10.1200/JCO.21.01874.
[9] A. Oaknin, B. Pothuri, L. Gilbert, et al., Safety, efficacy, and biomarker analyses of dostarlimab in patients with endometrial cancer: interim results of the phase I GARNET study, Clin. Cancer Res. (Jun 26 2023), https://doi.org/10.1158/1078-0432.CCR-22-3915.
[10] C. Luchini, F. Bibeau, M.J.L. Ligtenberg, et al., ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach, Ann. Oncol. : official J. European Soc. Med. Oncology 30 (8) (Aug 1 2019) 1232–1243, https://doi.org/10.1093/annonc/mdz116.
[11] P. Vikas, H. Messersmith, C. Compton, et al., Mismatch repair and microsatellite instability testing for immune checkpoint inhibitor therapy: ASCO endorsement of college of American pathologists guideline, J. Clin. Oncol. : official journal of the American Society of Clinical Oncology 41 (10) (Apr 1 2023) 1943–1948, https://doi.org/10.1200/JCO.22.02462.
[12] A.M. Mills, T.A. Longacre, Lynch syndrome screening in the gynecologic tract: current state of the art, Am. J. Surg. Pathol. 40 (4) (Apr 2016) e35–e44, https://doi.org/10.1097/PAS.0000000000000608.
[13] L. Li, F. Chen, J. Liu, et al., Molecular classification grade 3 endometrial endometrioid carcinoma using a next-generation sequencing-based gene panel, Front. Oncol. 12 (2022) 935694, https://doi.org/10.3389/fonc.2022.935694.
[14] A. Pasanen, M. Loukovaara, R. Butzow, Clinicopathological significance of deficient DNA mismatch repair and MLH1 promoter methylation in endometrioid endometrial carcinoma, Mod. Pathol. : official J. United States Canadian Academy of Pathology, Inc. 33 (7) (Jul 2020) 1443–1452, https://doi.org/10.1038/s41379-020-0501-8.
[15] A.O. Nygren, N. Ameziane, H.M. Duarte, et al., Methylation-specific MLPA (MS-MLPA): simultaneous detection of CpG methylation and copy number changes of up to 40 sequences, Nucleic Acids Res. 33 (14) (Aug 16 2005) e128, https://doi.org/10.1093/nar/gni127.
[16] H. Ellegren, Microsatellites: simple sequences with complex evolution, Nat. Rev. Genet. 5 (6) (Jun 2004) 435–445, https://doi.org/10.1038/nrg1348.
[17] A. Duval, R. Hamelin, Genetic instability in human mismatch repair deficient cancers, Ann. Genet. 45 (2) (Apr-Jun 2002) 71–75, https://doi.org/10.1016/s0003-3995(02)01115-2.
[18] K. Li, H. Luo, L. Huang, H. Luo, X. Zhu, Microsatellite instability: a review of what the oncologist should know, Cancer Cell Int. 20 (2020) 16, https://doi.org/10.1186/s12935-019-1091-8.
[19] A. Goel, T. Nagasaka, R. Hamelin, C.R. Boland, An optimized pentaplex PCR for detecting DNA mismatch repair-deficient colorectal cancers, PLoS One 5 (2) (Feb 24 2010) e9393, https://doi.org/10.1371/journal.pone.0009393.
[20] A. Umar, C.R. Boland, J.P. Terdiman, et al., Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability, J. Natl. Cancer Inst. 96 (4) (Feb 18 2004) 261–268, https://doi.org/10.1093/jnci/djh034.
[21] L. Libera, N. Sahnane, F. Pepe, et al., Critical aspects of microsatellite instability testing in endometrial cancer: a comparison study, Hum. Pathol. 128 (Oct 2022) 134–140, https://doi.org/10.1016/j.humpath.2022.07.014.
[22] S. Middha, L. Zhang, K. Nafa, et al., Reliable pan-cancer microsatellite instability assessment by using targeted next-generation sequencing data, JCO Precis Oncol (2017), https://doi.org/10.1200/PO.17.00084.
[23] L.G. Baudrin, J.F. Deleuze, A. How-Kit, Molecular and computational methods for the detection of microsatellite instability in cancer, Front. Oncol. 8 (2018) 621, https://doi.org/10.3389/fonc.2018.00621.
[24] I. Ukkola, P. Nummela, M. Kero, A. Ristimaki, Diagnostic performance of Idylla MSI test in colorectal cancer biopsies, Diagn. Pathol. 18 (1) (Mar 28 2023) 39, https://doi.org/10.1186/s13000-023-01328-6.
[25] I. Ruz-Caracuel, A. Lopez-Janeiro, V. Heredia-Soto, et al., Clinicopathological features and prognostic significance of CTNNB1 mutation in low-grade, early-stage endometrial endometrioid carcinoma, Virchows Arch. : an Int. J. pathology 479 (6) (Dec 2021) 1167–1176, https://doi.org/10.1007/s00428-021-03176-5.
[26] M. Mendiola, V. Heredia-Soto, I. Ruz-Caracuel, et al., Comparison of methods for testing mismatch repair status in endometrial cancer, Int. J. Mol. Sci. 24 (19) (Sep 23 2023), https://doi.org/10.3390/ijms241914468.

[27] C. Kopanos, V. Tsiolkas, A. Kouris, et al., VarSome: the human genomic variant search engine, Bioinformatics 35 (11) (Jun 1 2019) 1978–1980, https://doi.org/10.1093/bioinformatics/bty897.
[28] J.T. Robinson, H. Thorvaldsdottir, A.M. Wenger, A. Zehir, J.P. Mesirov, Variant review with the integrative genomics viewer, Cancer Res. 77 (21) (Nov 1 2017) e31–e34, https://doi.org/10.1158/0008-5472.CAN-17-0337.
[29] P. Gilson, C. Franczak, L. Dubouis, et al., Evaluation of KRAS, NRAS and BRAF hotspot mutations detection for patients with metastatic colorectal cancer using direct DNA pipetting in a fully-automated platform and Next-Generation Sequencing for laboratory workflow optimisation, PLoS One 14 (7) (2019) e0219204, https://doi.org/10.1371/journal.pone.0219204.
[30] J. Siemanowski, B. Schomig-Markiefka, T. Buhl, et al., Managing difficulties of microsatellite instability testing in endometrial cancer-limitations and advantages of four different PCR-based approaches, Cancers 13 (6) (Mar 12 2021), https://doi.org/10.3390/cancers13061268.
[31] Y. Wang, C. Shi, R. Eisenberg, C.L. Vnencak-Jones, Differences in microsatellite instability profiles between endometrioid and colorectal cancers: a potential cause for false-negative results? J. Mol. Diagn. : J. Mod. Dynam. 19 (1) (Jan 2017) 57–64, https://doi.org/10.1016/j.jmoldx.2016.07.008.
[32] G. Preud'homme, K. Duarte, K. Dalleau, et al., Head-to-head comparison of clustering methods for heterogeneous data: a simulation-driven benchmark, Sci. Rep. 11 (1) (Feb 18 2021) 4202, https://doi.org/10.1038/s41598-021-83340-8.
[33] W.K. Funkhouser Jr., I.M. Lubin, F.A. Monzon, et al., Relevance, pathogenesis, and testing algorithm for mismatch repair-defective colorectal carcinomas: a report of the association for molecular pathology, J. Mol. Diagn. : JMD. 14 (2) (Mar-Apr 2012) 91–103, https://doi.org/10.1016/j.jmoldx.2011.11.001.
[34] E. Mangold, C. Pagenstecher, W. Friedl, et al., Tumours from MSH2 mutation carriers show loss of MSH2 expression but many tumours from MLH1 mutation carriers exhibit weak positive MLH1 staining, J. Pathol. 207 (4) (Dec 2005) 385–395, https://doi.org/10.1002/path.1858.
[35] S.E. Hile, S. Shabashev, K.A. Eckert, Tumor-specific microsatellite instability: do distinct mechanisms underlie the MSI-L and EMAST phenotypes? Mutat. Res. 743–744 (Mar-Apr 2013) 67–77, https://doi.org/10.1016/j.mrfmmm.2012.11.003.
[36] A. Pecriaux, L. Favre, J. Calderaro, C. Charpy, J. Derman, A. Pujals, Detection of microsatellite instability in a panel of solid tumours with the Idylla MSI Test using extracted DNA, J. Clin. Pathol. 74 (1) (Jan 2021) 36–42, https://doi.org/10.1136/jclinpath-2020-206581.
[37] S.M. Evrard, E. Taranchon-Clermont, I. Rouquette, et al., Multicenter evaluation of the fully automated PCR-based Idylla EGFR mutation assay on formalin-fixed, paraffin-embedded tissue of human lung cancer, J. Mol. Diagn. : J. Mod. Dynam. 21 (6) (Nov 2019) 1010–1024, https://doi.org/10.1016/j.jmoldx.2019.06.010.
[38] S. Gatius, A. Velasco, M. Varela, et al., Comparison of the Idylla MSI assay with the Promega MSI Analysis System and immunohistochemistry on formalin-fixed paraffin-embedded tissue of endometrial carcinoma: results from an international, multicenter study, Virchows Arch. : an international journal of pathology 480 (5) (May 2022) 1031–1039, https://doi.org/10.1007/s00428-022-03291-x.
[39] I. Ukkola, P. Nummela, A. Pasanen, et al., Detection of microsatellite instability with Idylla MSI assay in colorectal and endometrial cancer, Virchows Arch. : an international journal of pathology 479 (3) (Sep 2021) 471–479, https://doi.org/10.1007/s00428-021-03082-w.
[40] P. Rafaniello-Raviele, I. Betella, A. Rappa, et al., Microsatellite instability evaluation: which test to use for endometrial cancer? J. Clin. Pathol. 76 (1) (Jan 2023) 29–33, https://doi.org/10.1136/jclinpath-2021-207723.
[41] F. Dedeurwaerdere, K.B. Claes, J. Van Dorpe, et al., Comparison of microsatellite instability detection by immunohistochemistry and molecular techniques in colorectal and endometrial cancer, Sci. Rep. 11 (1) (Jun 18 2021) 12880, https://doi.org/10.1038/s41598-021-91974-x.
[42] X. Wu, O. Snir, D. Rottmann, S. Wong, N. Buza, P. Hui, Minimal microsatellite shift in microsatellite instability high endometrial cancer: a significant pitfall in diagnostic interpretation, Mod. Pathol. : an official journal of the United States and Canadian Academy of Pathology, Inc. 32 (5) (May 2019) 650–658, https://doi.org/10.1038/s41379-018-0179-3.