Journal of
Molecular Pathology

MDPI

Review

Molecular Profiling of H-MSI/dMMR/for Endometrial Cancer Patients: "New Challenges in Diagnostic Routine Practice"

Riccardo Adorisio [1], Giancarlo Troncone [2,*,†], Massimo Barberis [3,†] and Francesco Pepe [2]

[1] Division of Pathology, European Institute of Oncology (IEO), Istituto Di Ricovero E Cura A Carattere Scientifico (IRCCS), Via Giuseppe Ripamonti 435, 20141 Milan, Italy; riccardo.adorisio@ieo.it
[2] Department of Public Health, Federico II University of Naples, Via S. Pansini, 5, 80131 Naples, Italy; francesco.pepe4@unina.it
[3] Division of Advanced Molecular Diagnostics (DIMA), European Institute of Oncology IRCCS, Via Giuseppe Ripamonti 435, 20141 Milan, Italy; massimo.barberis@ieo.it
* Correspondence: giancarlo.troncone@unina.it; Tel.: +39-0817-463-674
† These authors contributed equally to this work.

Abstract: Endometrial cancer (EC) represents one of the most newly diagnosed cancers across gynecological malignancies. In particular, a plethora of risk factors (both biological and lifestyle-related) drastically impact the incidence rate of novel diagnosis accounting for 8300 cases/year. In the recent era of precision medicine EC molecular classification, integrating ESGO/ESTRO/ESP guidelines, four distinct diagnostic groups have been established including POLE-mutant (POLE-pos); High-instability MSI (H-MSI)–MMR-deficient (MMR-d); p53-abnormal (p53abn); and non-specific molecular profile (NSMP), also known as p53-wild-type EC patients on the basis of clinically relevant emerging biomarkers. In addition, molecular testing also plays a pivotal role in defining the best therapeutical option. In this scenario, the European Society for Medical Oncology (ESMO) recommended d-MMR/MSI-H status evaluation in the diagnostic workflow of Lynch syndrome or selecting EC patients that could benefit from immune checkpoint inhibitors (ICIs). Although immunohistochemistry (IHC) is considered the gold standard approach for d-MMR profiling, a series of molecular PCR-based techniques have rapidly developed to integrate H-MSI status in routine practice. Here, we technically overviewed the most relevant commercially available diagnostic assays for the determination of the H-MSI/dMMR status in EC patients.

Keywords: endometrium; molecular pathology; point-of-care testing

Citation: Adorisio, R.; Troncone, G.; Barberis, M.; Pepe, F. Molecular Profiling of H-MSI/dMMR/for Endometrial Cancer Patients: "New Challenges in Diagnostic Routine Practice". *J. Mol. Pathol.* **2024**, *5*, 187–198. https://doi.org/10.3390/jmp5020012

Academic Editor: Giuseppe Banna

Received: 7 March 2024
Revised: 4 April 2024
Accepted: 22 April 2024
Published: 24 April 2024

1. Introduction

Endometrial cancer (EC) is the most prevalent gynecological tumor in Europe and in the United States, with respectively 130,051 and 101,672 cases [1,2]. In Italy, it represents the third most common cancer in women aged between 50 and 69 years, accounting for approximately 8300 new cases each year [3]. The prevalence of endometrial cancer has surged by 132.0% in the last three decades, mirroring the increasing occurrence of its associated risk factors. In contrast, mortality rates have experienced a decline of 15.0% over the same time [4]. Risk factors for endometrial cancer include extended exposure to unopposed estrogen, often resulting from nulliparity and infertility associated with polycystic ovarian syndrome or tamoxifen use, as well as obesity and hyperinsulinemia [5–7]. Particularly, among the 20 most common tumor types, endometrial cancer exhibits the most significant correlation with obesity. For each 5 kg/m^2 increase in BMI, there is a 54.0% higher risk of developing this type of cancer [8]. Obesity creates a hyper-estrogenic condition as adipose tissue converts adrenal androgens into estrogen through peripheral aromatization. Estrogen promotes the proliferation of the endometrium, while cyclical progesterone and regular menstrual shedding maintain endometrial health during reproductive years. In postmenopausal women, the deficiency of natural progesterone

contributes to an unopposed estrogen excess [9]. Endometrial tumors originate from epithelial cells of Müllerian origin [10]. There are several histological types of endometrial carcinomas with different incidences. Particularly, they were grouped into endometrioid (Type I) and non-endometrioid (Type II) tumor types showing specific morphological and clinical patterns [11]. Endometrioid adenocarcinoma represents the most common estrogen-related histological types, accounting for 75–80% of cases [12]. Most patients develop an early-stage tumor, confined to the uterus, usually with a favorable prognosis [13]. In the era of precision medicine, rapidly emerging technical approaches comprehensively encourage the molecular evaluation of the genetic basis of EC. The Cancer Genome Atlas (TCGA) identified four distinct EC subgroups with clinic-pathological and molecular specific features. This effort has revolutionized the clinical administration of EC patients in accordance with the molecular signature identifying molecular drivers of each histological subtype [14]. As a result, it has paved the way for precision oncology approaches to clinically administrate EC patients. In clinical practice, TCGA molecular groups can be accurately stratified by evaluating three molecular markers: *POLE* (Polymerase DNA) Epsilon, Catalytic Subunit) hotspot mutations, *p53* (Tumor Protein P53) expression level, and mismatch repair (MMR) protein status. Approaching these biomarkers in clinical practice, a novel diagnostic algorithm enables EC patients' classification in four distinct molecular groups: POLE-mutant (POLE-pos); MMR-deficient (MMR-d); p53-abnormal (p53abn); and non-specific molecular profile (NSMP), also known as p53-wild-type [15]. This classification has been integrated into ESGO/ESTRO/ESP guidelines for the management of EC patients [16] establishing the clinical role of this novel approach available in routine practice. In this scenario, Betella et al. proposed a novel strategical algorithm based on ESGO/ESTRO/ESP 2020 guidelines that allows for the molecular stratification of EC patients adopting optimized technical approaches [17]. Comparing the novel risk stratification system with the approved guidelines, a discordance rate of 6.8% (;CI 4.2% to 10.5%; 19 out of 278 patients) was observed. Particularly, this novel algorithm decreased the POLE sequencing analysis and *p53* immunohistochemistry rate by 67.0% and 27.0, respectively, optimizing the technical workflow routinely available for molecular analysis. The mismatch repair (MMR) system is a well-preserved mechanism that plays a crucial role in restoring mismatch errors. Genetic (somatic and germline) and epigenetic changes result in a deficient MMR (dMMR) system promoting the activation of error-prone DNA repair systems [18]. Among them, the vast majority of EC marked by the dMMR status is sporadic, while a low percentage of dMMR EC patients (3.0%) highlight hereditary lineage, associated with Lynch syndrome (LS), an autosomal dominant inherited disease, caused by germline mutations in MMR genes: *MLH1*, *MSH2*, *MSH6*, and *PMS2* [19,20]. The main effect of dMMR status occurs in microsatellite sequences. Microsatellites are short tandem mono or dinucleotide repeated sequences found in coding and non-coding genomic regions. These sequences may be affected by a variation of the number of repetitions during DNA replication. In the instance of a dysfunctional MMR complex, microsatellite sequences may not be restored. This event is considered indirect evidence of a dMMR status resulting in microsatellite instability (MSI) [21]. Of note, EC patients are diagnosed with severe MSI status (H-MSI) in 20–30% of cases. Recently, d-MMR/MSI-H status was approved to elect EC patients that could benefit from immune checkpoint inhibitors (dostarlimab) [22,23]. In 2019, the European Society for Medical Oncology (ESMO) recommended d-MMR/MSI-H evaluation as diagnostic tool in Lynch syndrome patients adopting immunohistochemistry (IHC) or molecular Polymerase chain reaction-based (PCR-based) tests to detect d-MMR and MSI-H status, respectively [24]. As a matter of fact, MSI status evaluation has been routinely carried out in microfluidic systems after approaching PCR-based techniques for the amplification of loci of the Bethesda panel (*BAT-25*, *BAT26*, *D2S123*, *D5S346*, and *D17S250*) [25]. Although there is a widespread diffusion in clinical practice of PCR-based approaches built on the Bethesda panel, MSI testing in EC patients highlighted several technical limitations in terms of the MSI-H status detection rate. In this scenario, optimized panels including mononucleotide repetitions have been developed for improving the de-

tection rate of MSI-H status in EC patients [26]. Currently, several commercially available panels, like LMR MSI Analysis System (Promega), able to improve technical sensitivity and specificity in detecting mononucleotide markers in EC patients, have been developed [27]. Of note, these approaches are affected by their extensive time-consuming nature as well as necessitate the availability of both normal and tumor tissues to compare molecular profiles for MSI status detection. Here, our aim consists of the technical analysis of several commercially available kits for the determination of MSI/dMMR status evaluation in the diagnostic practice of EC patients (Table 1).

Table 1. Technical evaluation of MSI/dMMR testing strategies routinely available for EC patients' stratification.

Assay	Analyzed Loci	Advantages	Disadvantages
IHC	4 MMR proteins (MLH1/MSH2/MSH6/PMS2)	- High specificity and sensitivity - Rapid turnaround time (4–6 h) - Feasible in samples with less than 20% neoplastic cell content - Not expensive	- Requires individual processing of four slides for MMR protein staining - Requires experienced pathologist for result interpretation - Heterogeneous MMR protein expression - Possible false positive results due to pre-analytic issues or absence of evident loss of expression due to intact immunoreactivity
PENTAPLEX BETHESDA PANEL ASSAY	5 microsatellite markers (BAT25, BAT26, D2S123, D5S346, and D17S250)	- Low-cost analysis - Fast turnaround time (<5 h) - High reproducibility	- Requires samples with at least 20% neoplastic cellularity - No provided indication about MMR genes to investigate - Selectivity for few cancer types due to limited number of targets investigated - Possibility of detecting occasional false positive results, owed to microsatellite polymorphisms
TITANO MSI	10 microsatellite markers (BAT25, BAT26, D2S123, D17S250, D5S346, BAT40, D18S58, NR21, NR24, and TGFβRII)	- Multiplexed PCR assays - Low-cost analysis - Fast turnaround time for result (<5 h)	- Required matched normal tissue - Not designed for EC
OncoMate^{TM} MSI Dx ANALYSIS SYSTEM	7 microsatellite markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27, Penta C, and Penta D)	- Multiplexed PCR assays - Rapid turnaround time (<5 h) - Low-cost analysis - Better sensitivity than PentaPlex Bethesda panel assay	- No provided indication about MMR genes to investigate - Required a matched normal tissue - Not designed for EC
LMR MSI ANALYSIS SYSTEM	8 microsatellite markers (BAT-25, BAT-26, MONO-27, NR-21, BAT-52, BAT-56, BAT-59, and BAT-60)	- Multiplexed PCR assays - Turnaround time (<5 h) - Low-cost analysis - Better sensitivity than PentaPlex Bethesda panel assay and OncoMate^{TM} MSI Dx Analysis System - LMR markers are more sensitive in EC	- No provided indication about MMR genes to investigate - Required matched normal tissue - Not designed for EC

Table 1. *Cont.*

Assay	Analyzed Loci	Advantages	Disadvantages
EasyPGX® ready MSI KIT	8 microsatellite markers (BAT-25, BAT-26, NR-21, NR-22, NR-24, NR-27, CAT-25, and MONO-27)	- No requirement of paired normal tissue for MSI analysis - Analysis can be performed using FFPE tissue samples or blood samples - Highly reproducible - High sensitivity - Turnaround time for result (<4 h) - Hands-on time is less than 15 min	- No provided indication about MMR genes to investigate
Idylla®MSI TEST	7 microsatellite markers (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, and SULF2)	- Multiplexed PCR assays - Low-cost analysis - Highly reproducible - Turnaround time 150 min - Hands-on time is less than 5 min - No required previous DNA extraction - No required paired normal tissue for MSI analysis	- No provided indication about MMR genes to investigate - Required at least 20% neoplastic cells
ddPCR MSI KIT Bio-Rad®	5 microsatellite markers (BAT25, BAT26, Mono27, NR21, and NR24)	- Low-cost analysis - No required paired normal tissue for MSI analysis - Analysis can be also performed on liquid biopsy - Turnaround time (<5 h)	- No provided indication about MMR genes to investigate - Lack of standardization for results' interpretation

2. Immunohistochemistry

IHC represents the most available assay in routine practice for dMMR analysis thanks to a sustainable technical cost [28]. Basically, IHC workflow comprises utilizing a staining evaluation of four proteins of the MMR complex (MLH1, MSH3, MSH6, PMS2). The positive signal is inspected by an expert pathologist able to interpret the expression level of each target. A crucial point of IHC analysis is represented by the positive signal of the internal control expressed in lymphocytes and other non-neoplastic cells. IHC may be considered a challenging approach in terms of artifacts' signal and intra-operator variability. Controversial results are literally reported on the concordance rate between IHC and PCR-based testing strategies. Previous studies highlighted a concordance rate of 95.0% between IHC and PCR-based MSI testing [29]. Stelloo et al. also demonstrated a not negligible (41.6%) rate of ambiguous cases not adequately interpretable with IHC [30]. In this scenario, Malapelle et al. evaluated several technical parameters of diagnostic samples simultaneously elected to molecular test and IHC analysis. Among them, the DNA fragmentation index drastically impacted the concordance rate between amplicon-based approaches and the IHC system [31]. At the sight of these aspects, some laboratories implemented a combined diagnostic approach integrating gold standard IHC with novel molecular testing strategies in order to improve the number of diagnostic cases correctly stratified [32].

3. Idylla™ MSI Assay

The Idylla Biocartis MSI test (Biocartis NV, Mechelen, Belgium) consists of a fully automatized system enabled to assess MSI analysis. Briefly, the Idylla Biocartis MSI test is carried out analyzing seven different loci (*ACVR2A*, *BTBD7*, *DIDO1*, *MRE11*, *RYR3*, *SEC31A*, and *SULF2*) [33]. A fluorescent signal deriving from optimized probes and primers (MNAzymes PlexPrimers) allows for the detection of monomorphic variation in targeted regions. In comparison with standardized primers, MNAzymes improves testing accuracy

in terms of specificity and sensitivity [34]. This approach allows for molecular analysis starting from extracted DNA or formalin-fixed, paraffin-embedded (FFPE) tissue specimens decreasing laborious and time-consuming manual procedures [35,36]. These advantages improve the test's accuracy and reliability, enabling precise MSI status determination. Previous studies focused on the technical evaluation of the Idylla MSI test in a retrospective series of EC patients [37]. Of note, a technical sensitivity of 72.7% and specificity of 100% were assessed in a series of n = 108 diagnostic routine samples from EC patients. In addition, a concordance rate of 82.7% was also observed comparing the Idylla MSI test with the IHC approach. Remarkably, by selecting samples with ≥30.0% of neoplastic cell percentage, the concordance rate improved.

4. Pentaplex Bethesda Panel Assay

The Pentaplex Bethesda panel covers a set of five mono- and dinucleotide microsatellite markers (*BAT25*, *BAT26*, *D2S123*, *D5S346*, and *D17S250*) developed by Bethesda consortium for the analysis of MSI status in colorectal cancer patients (CRC) [25,38]. This approach is set on the PCR amplification of the microsatellite loci followed by fragment length analysis enabled to inspect the amplification profile and detect any changes in the lengths of the repeated sequences. Unfortunately, this assay requires a simultaneous analysis of non-tumoral tissue in order to compare the amplification profile with the tumor sample inspecting variations in terms of length for amplified loci [39]. To date, the Bethesda panel is a widely used diagnostic tool considered a referral method for MSI testing in CRC patients thanks to diagnostic, prognostic, and guiding therapy obtained for diagnostic routine cases. Conversely, technical limitations may occur when evaluating EC samples due to dinucleotide repeated markers covered by this panel. Dinucleotide markers included in the Bethesda panel (*D2S123*, *D17S250*, and *D5S546*) exhibit high polymorphic and not easily interpretable alterations causing a high false negative rate in MSI analysis [25,40]. In this scenario, optimized microsatellite panels, integrating mononucleotide repeated sequences, may be considered a reliable diagnostic strategy to detect MSI-H in EC patients.

5. OncoMate™ MSI Dx Analysis System

The OncoMate™ MSI Dx Analysis System (Promega, Fitchburg, MA, USA) is a PCR-based fragment-sizing test developed to evaluate MSI status from diagnostic routine specimens [41]. This approach requires the PCR amplification of both tumor and non-tumor FFPE tissue samples comparing the microsatellite profile on proprietary software. Briefly, the diagnostic workflow is set on the quantitative PCR amplification of microsatellite markers evaluated on the capillary electrophoresis system [37]. The OncoMate™ MSI Dx Analysis System targets seven microsatellite markers: five mononucleotide repeat markers (*BAT-25*, *BAT-26*, *NR-21*, *NR-24*, and *MONO-27*) and two pentanucleotide repeat markers (Penta C and Penta D) showing a high sensitivity and specificity in clinical patients' administration. Instead, pentanucleotide repeat markers are examined to confirm the identity match between DNA samples from the normal and tumor tissues [37]. In a multicenter trial, Gatius et al. analyzed n = 242 FFPE EC samples collected by seven referral centers. They compared the Idylla™ MSI assay, Promega™ MSI Analysis System, and IHC for MMR proteins in terms of the MMR/MSI detection rate and technical costs supporting the analytical approach. The results showed a concordance rate of 88.5% and 89.9% between IHC and the Promega™ MSI assay and Idylla™ and Promega™ MSI assays, respectively. Of note, Invalid results were identified in 5.4% of cases adopting the Promega™ MSI assay in comparison with 7.0% of the IHC approach. The concordance rate between the Idylla™ MSI assay and Promega™ MSI assay improved to 91.2% setting an instability cutoff of 0.3 [41].

6. LMR MSI Analysis System

The LMR MSI Analysis System (Promega) represents a PCR-based technique able to detect MSI status in diagnostic specimens from solid tumor patients [42,43]. This tool is based on the analysis of four gold standard MSI markers (*BAT-25*, *BAT-26*, *MONO-27*

and *NR-21*) integrated with long mononucleotide repeat (LMR) markers (*BAT-52*, *BAT-56*, *BAT-59*, and *BAT-60*) improving analytical performance for the detection of MSI status. These markers enhance the capability to detect MSI status, particularly, and they showed greater sensitivity for detecting MSI in EC [27,43]. In a retrospective cohort of solid tumor patients (including CRC, EC), Lin et al. compared the MSI-H detection rate by adopting LMR MSI analysis with an IHC signal. Of note, the results showed a technical sensitivity and specificity of 98.0% and 100.0%, respectively, in EC patients [44]. In another experience, a technical sensitivity of 98.0% and 100.0%, respectively, was reached on a retrospective series of EC patients [45].

7. Titano MSI Test

The Titano MSI kit enables the assessment of MSI status inspecting the fluorescent signal of multiplex amplified loci on the Sanger sequencing platform [46]. This approach has proven technical advantages in terms of the detection of small repeated sequences across 10 distinct molecular targets (*BAT25*, *BAT26*, *D2S123*, *D17S250*, *D5S346*, *BAT40*, *D18S58*, *NR21*, *NR24*, and *TGFβRII*) improving the detection rate of H-MSI cases in clinical practice [46]. This tool requires an amplification profile of tumor and matched non-tumor derived samples in order to evaluate MSI status. Starting from 20 ng of the extracted DNA, this system accurately analyzed repeated sequences' variations across different molecular targets [31,33]. Of note, the Titano MSI kit was also approached to solve discordant cases on a retrospective series of n = 20 EC patients evaluated by IHC, a microfluidic system, and a semi-automatized RT-PCR system demonstrating a high concordance rate with molecular systems [31,33].

8. EasyPGX® Ready MSI Kit

The EasyPGX® ready MSI kit represents a semi-automatized, analysis software integrated technical strategy for the determination of MSI status from several diagnostic specimens. From a technical point of view, this platform directly analyzes n = 8 monomorphic and mononucleotide microsatellite markers (*BAT-25*, *BAT-26*, *NR-21*, *NR-22*, *NR-24*, *NR-27*, *CAT-25*, and *MONO-27*) employing fluorescent labeled probes. The manufacturer's instructions assess that the minimal input required is 10–20 ng for blood and 10–100 ng for FFPE tissue samples for the MSI status [47]. MSI status is determined by comparing the melt curve of each marker with a respectively stable control sample. A dedicated analysis software integrates a fluorescent signal in an easily managed report where the MSI score is automatically calculated. Thanks to easily managing analytical procedures, the Easy PGX MSI kit is routinely employed in the diagnostic workflow of Italian institutions (26.0%) where it has been elected as a referral diagnostic tool for MSI analysis in EC patients as shown by data of an Italian survey [48]. Several studies demonstrated that this approach is suitable for MSI status evaluation in EC patients. As regards, Libera et al. identified MSI-H and MSS status in 19 out of 31 (61.3%) and 25 out of 25 (100.0%) dMMR and pMMR EC patients, respectively [37].

9. ddPCR Microsatellite Instability (MSI) Kit

Overall, the ddPCR Microsatellite Instability (MSI) Kit (Hercules, CA, USA) may be considered a commercially available RT-PCR-based assay able to accurately detect MSI status from 2 ng of extracted DNA inspecting deletions and insertions in five mononucleotide microsatellite markers from plasma or tissue samples [49,50]. A previous validation study on a series of n = 15 EC patients widely demonstrated that this approach is a technically reliable diagnostic system that aimed for MSI status evaluation (a concordance rate of 100.0% in comparison with the IHC gold standard method) [46] (Figure 1).

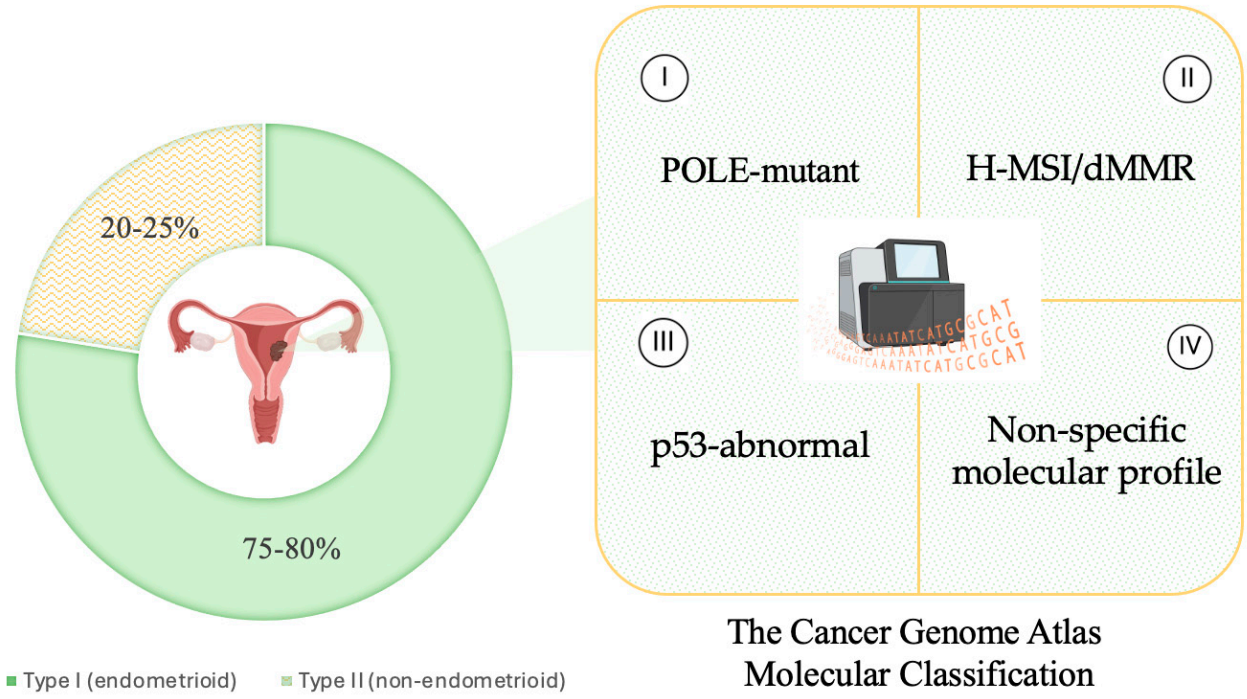

Figure 1. Representation of histological type and TCGA molecular groups of EC patients.

10. Advantages and Disadvantages

To date, MMR/MSI status has rapidly revolutionized the clinical landscape of EC patients. In this scenario, dostarlimab (immune checkpoint inhibitor) was approved to treat H-MSI/d-MMR EC patients thanks to a statistically significant benefit in terms of progression-free survival at 24 months (61.4%, 95% confidence interval [CI], 46.3 to 73.4 versus 15.7% (95% CI, 7.2 to 27.0) and overall survival at 24 months (71.3% (95% CI, 64.5 to 77.1) with dostarlimab compared with 56.0% (95% CI, 48.9 to 62.5) of the placebo group [51]. At the sight of these aspects, H-MSI/d-MMR became pivotal in the clinical management of EC patients. Immunohistochemistry (IHC) and PCR-based assays carried out on tumor tissue samples, including biopsies or surgical resections, are considered the gold standard approach for determining MSI/dMMR status. Immunohistochemistry (IHC) highlights a notable technical sensitivity and specificity in the predominant LS-associated cancers, evaluating the expression of the four main MMR proteins [52,53]. Moreover, as a reliable alternative to traditional IHC-based testing, MSI-PCR methods involving the PCR amplification of microsatellite regions, followed by capillary electrophoresis, are routinely adopted in diagnostic practice integrating IHC analysis [54]. To date, IHC and MSI-PCR techniques exhibit a comparable detection rate inspecting H-MSI/dMMR status in colorectal and endometrial cancers [55–58]. Despite the proven availability of traditional MSI-PCR methods based on the Bethesda panel, several commercial PCR-based tests have gained market prominence, distinguishing themselves from the older ones due to an extensive reference range including monomorphic loci molecularly relevant in EC patients. IHC is considered the gold standard testing strategy for identifying MSI in EC patients thanks to easily managing analytical procedures, low TAT (about 4–6 h), and reduced technical costs. Furthermore, IHC shows robust results even if the starting diagnostic sample is featured by a neoplastic cell content of 20.0%. On the other hand, IHC requires correct pre-analytical procedures decreasing artifacts at morphological analysis as well as an experienced pathologist for result interpretation [59]. Other limitations depending on the biological mechanisms of the tumor concern heterogeneous MMR protein expression that drastically impacts reproducible results. Remarkably, false positive results may also derive from pre-analytic issues, while false negative results are caused by the absence of the evident loss of expression due to the intact immunoreactivity signal [24]. The Pentaplex

Bethesda panel is considered the first widely accepted PCR-based technique for the detection of MSI status. Similarly, PCR-based systems show the low TAT (less than 5 h), saving cost approaches, and high reproducibility rate. Despite the evident benefits of the method, PCR-based systems require samples with 20% neoplastic cells [60]. Both the OncoMate™ MSI Dx Analysis System and the LMR MSI Analysis System tests demonstrated technical advantages in terms of the rapid turnaround time (less than 5 h) and low analysis costs. Furthermore, these assays show a decreasing rate of false positive results in comparison with the Bethesda-based assays [61,62]. Like the Bethesda panel, they do not provide indications regarding MMR genes to investigate. Moreover, they have not been certified CE-IVD for microsatellite analysis in EC. Regardless, the LMR MSI Analysis System, owing to the LMR markers, produces more pronounced fragment length changes, improving the detection of microsatellite instability [27,63]. All these approaches need a normal tissue profile to evaluate MSI status.

The Titano MSI kit is versatile and extensively adopted in diagnostic routine practice. Although this assay has been not specifically optimized for EC analysis, it highlights consistent agreement with other techniques [64]. Nevertheless, the Titano MSI test represents a low-cost multiplexed analysis, with a clinical useful turnaround time (5 h). Unlike the Titano MSI test, the EasyPGX® ready MSI kit does not require a paired normal tissue for MSI analysis. The analysis can be performed using FFPE tissue samples or blood samples. The turnaround time of this test from sample to result is less than 4 h and hands-on time is no more than 15 min. As long as there is a presence of mononucleotide repeats, the assay demonstrates high reproducibility and sensitivity [63]. The Idylla MSI test is a multiplexed, highly reproducible, and low-cost method. This approach does not require a paired normal tissue sample to successfully interpret molecular data. Furthermore, this assay is considered a rapid (from sample to result of 150 min) and minimal hands-on time of about 5 min technical strategy. A limitation of the technique is the technical requirement of at least 20% neoplastic cellularity to validate molecular interpretation [50]. Although it has been designed for colorectal cancer samples, the Idylla MSI test demonstrated high analytical performance in EC cases [64]. Given the current inclination towards molecular tumor diagnostics relying on ctDNA analysis and the multifaceted implications of MSI in cancer management, there is an increasing demand for innovative methods for diagnosing MSI in blood samples. A PCR-based method that allows for MSI analysis starting from blood samples is represented by the ddPCR Microsatellite Instability (MSI) Kit. It represents a cost-effective solution with a fast turnaround time of less than 5 h. The Idylla MSI test and the EasyPGX® ready MSI kit do not require a matched normal sample and exhibit high sensitivity and an elevated accuracy of MSI evaluation in EC liquid biopsy samples. Also in this case, it does not provide information about altered MMR genes. In addition, there is a lack of standardization for the results' interpretation. In terms of saving technical costs, Orellana et al. highlighted that the molecular testing strategy is cost-effective in comparison with the no testing approach (USD 100,000/QALY) including in this statistical evaluation all clinically promising biomarkers for EC patients. Particularly, the IHC-based approach, routinely adopted in diagnostic routine practice, represents a cost-effective testing strategy compared with molecular techniques, whereas molecular testing approaches are sustainable setting a willingness-to-pay threshold of USD 100,000/QALY [65]. At the sight of these critical aspects, the College of American Pathologists established a consensus panel able to overcome pre-analytical and analytical bias in H-MSI/dMMR profile evaluation for EC patients. Particularly, EC patients eligible to ICIs in accordance with MSI status should be tested adopting an integrated workflow based on MMR-IHC MSI by PCR or next-generation sequencing (NGS) for the detection of DNA mismatch repair defects [66]. Taking into account technical opening challenges in detecting MSI status, NGS platforms enable the simultaneous analysis of several loci potentially reflecting genomic instability among different tumor types. As shown by Bartels et al., a technical sensitivity and specificity of 88.6% and 95.2%, respectively, were calculated on a series of EC patients previously tested with standardized approaches [67]. Although this comprehensive approach allows

for the detection of H-MSI status across several types of histological tumors; optimized bioinformatic pipelines are required to implement NGS based on the diagnostic routine practice of EC patients [68].

11. Conclusions

Assessing MSI status in EC holds significant clinical relevance due to its diagnostic, prognostic, and therapeutic implications. IHC and canonical PCR-based methods are considered the gold standard for determining MSI/dMMR status in EC patients. Nevertheless, alternative strategies, like ddPCR assays, show potential clinical implementation determining MSI/dMMR status. A plethora of commercially available PCR-based assays are available in diagnostic routine practice. Among these ones, different pre-analytical and analytical manufacturer procedures may be observed. The latest point clearly demonstrates that harmonized trials should be approached to solve open challenges about the implementation of these assays in clinical practice. Since the sensitivity of PCR-based assays is greater in colorectal cancers compared to endometrial cancers, an integrated diagnostic algorithm combining IHC and molecular testing strategies increases the successful rate in the clinical stratification of solid tumor patients.

Author Contributions: Conceptualization: R.A., G.T., M.B. and F.P. Methodology: R.A., G.T., M.B. and F.P. Software: R.A., G.T., M.B. and F.P. Validation: R.A., G.T., M.B. and F.P. Formal analysis: R.A., G.T., M.B. and F.P. Investigation: R.A., G.T., M.B. and F.P. Resources: R.A., G.T., M.B. and F.P. Data curation: R.A. and F.P. Writing—original draft preparation: R.A. and F.P. Writing—review and editing: M.B. and G.T. Visualization: all authors. Supervision: M.B. and G.T. Project administration: M.B. and G.T. Funding acquisition: M.B. and G.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: M.B. has received honoraria for consulting, an advisory role, speakers' bureau, travel, accommodation, expenses from MSD Oncology, Roche/Genetech, Astra Zeneca, Thermofisher Scientific, GSK, and Illumina unrelated to the current work. G.T. reports personal fees (as speaker bureau or advisor) from Roche, MSD, Pfizer, Boehringer Ingelheim, Eli Lilly, BMS, GSK, Menarini, AstraZeneca, Amgen, and Bayer, unrelated to the current work. F.P. reports personal fees (as speaker bureau or advisor) from Menarini unrelated to the current work.

References

1. Moreira, A.S.; Ribeiro, V.; Aringhieri, G.; Fanni, S.C.; Tumminello, L.; Faggioni, L.; Cioni, D.; Neri, E. Endometrial Cancer Staging: Is There Value in ADC? *J. Pers. Med.* **2023**, *13*, 728. [CrossRef] [PubMed]
2. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef] [PubMed]
3. AIOM-Airtum. I Numeri del Cancro in Italia 2020. Available online: https://www.aiom.it/i-numeri-del-cancro-in-italia/ (accessed on 1 March 2024).
4. Gu, B.; Shang, X.; Yan, M.; Li, X.; Wang, W.; Wang, Q.; Zhang, C. Variations in incidence and mortality rates of endometrial cancer at the global, regional, and national levels, 1990–2019. *Gynecol. Oncol.* **2021**, *161*, 573–580. [CrossRef] [PubMed]
5. Shetty, C.; Rizvi, S.; Sharaf, J.; Williams, K.D.; Tariq, M.; Acharekar, M.V.; Saldivia, S.E.G.; Unnikrishnan, S.N.; Chavarria, Y.Y.; Akindele, A.O.; et al. Risk of Gynecological Cancers in Women With Polycystic Ovary Syndrome and the Pathophysiology of Association. *Cureus* **2023**, *15*, e37266. [CrossRef] [PubMed]
6. Raglan, O.; Kalliala, I.; Markozannes, G.; Cividini, S.; Gunter, M.J.; Nautiyal, J.; Gabra, H.; Paraskevaidis, E.; Martin-Hirsch, P.; Tsilidis, K.K.; et al. Risk factors for endometrial cancer: An umbrella review of the literature. *Int. J. Cancer* **2018**, *145*, 1719–1730. [CrossRef] [PubMed]
7. Braun, M.M.; Overbeek-Wager, E.A.; Grumbo, R.J. Diagnosis and Management of Endometrial Cancer. *Am. Fam. Phys.* **2016**, *93*, 468–474.

8. Crosbie, E.J.; Kitson, S.J.; McAlpine, J.N.; Mukhopadhyay, A.; Powell, M.E.; Singh, N. Endometrial cancer. *Lancet* **2022**, *399*, 1412–1428. [CrossRef] [PubMed]
9. Kaaks, R.; Lukanova, A.; Kurzer, M.S. Obesity, endogenous hormones, and endometrial cancer risk: A synthetic review. *Cancer Epidemiol. Biomark. Prev.* **2002**, *11*, 1531–1543.
10. Merritt, M.A.; Cramer, D.W. Molecular pathogenesis of endometrial and ovarian cancer. *Cancer Biomark.* **2011**, *9*, 287–305. [CrossRef]
11. Llobet, D.; Pallares, J.; Yeramian, A.; Santacana, M.; Eritja, N.; Velasco, A.; Dolcet, X.; Matias-Guiu, X. Molecular pathology of endometrial carcinoma: Practical aspects from the diagnostic and therapeutic viewpoints. *J. Clin. Pathol.* **2008**, *62*, 777–785. [CrossRef]
12. Höhn, A.K.; Brambs, C.E.; Hiller, G.G.R.; May, D.; Schmoeckel, E.; Horn, L.-C. 2020 WHO Classification of Female Genital Tumors. *Geburtshilfe Frauenheilkd.* **2021**, *81*, 1145–1153. [CrossRef] [PubMed]
13. Gordhandas, S.; Zammarrelli, W.A.; Rios-Doria, E.V.; Green, A.K.; Makker, V. Current Evidence-Based Systemic Therapy for Advanced and Recurrent Endometrial Cancer. *J. Natl. Compr. Cancer Netw.* **2023**, *21*, 217–226. [CrossRef] [PubMed]
14. Kandoth, C.; Schultz, N.; Cherniack, A.D.; Akbani, R.; Liu, Y.; Shen, H.; Robertson, A.G.; Pashtan, I.; Shen, R.; Benz, C.C.; et al. Integrated genomic characterization of endometrial carcinoma. *Nature* **2013**, *497*, 67–73. [CrossRef] [PubMed]
15. Talhouk, A.; McConechy, M.K.; Leung, S.; Yang, W.; Lum, A.; Senz, J.; Boyd, N.; Pike, J.; Anglesio, M.; Kwon, J.S.; et al. Confirmation of ProMisE: A simple, genomics-based clinical classifier for endometrial cancer. *Cancer* **2017**, *123*, 802–813. [CrossRef]
16. Concin, N.; Matias-Guiu, X.; Vergote, I.; Cibula, D.; Mirza, M.R.; Marnitz, S.; Ledermann, J.; Bosse, T.; Chargari, C.; Fagotti, A.; et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. *Int. J. Gynecol. Cancer* **2021**, *31*, 12–39. [CrossRef]
17. Betella, I.; Fumagalli, C.; Raviele, P.R.; Schivardi, G.; De Vitis, L.A.; Achilarre, M.T.; Aloisi, A.; Garbi, A.; Maruccio, M.; Zanagnolo, V.; et al. A novel algorithm to implement the molecular classification according to the new ESGO/ESTRO/ESP 2020 guidelines for endometrial cancer. *Int. J. Gynecol. Cancer* **2022**, *32*, 993–1000. [CrossRef] [PubMed]
18. Olave, M.C.; Graham, R.P. Mismatch repair deficiency: The what, how and why it is important. *Genes Chromosom. Cancer* **2021**, *61*, 314–321. [CrossRef]
19. Cerretelli, G.; Ager, A.; Arends, M.J.; Frayling, I.M. Molecular pathology of Lynch syndrome. *J. Pathol.* **2020**, *250*, 518–531. [CrossRef] [PubMed]
20. Post, C.C.; Stelloo, E.; Smit, V.T.; Ruano, D.; Tops, C.M.; Vermij, L.; Rutten, T.A.; Jürgenliemk-Schulz, I.M.; Lutgens, L.C.; Jobsen, J.J.; et al. Prevalence and Prognosis of Lynch Syndrome and Sporadic Mismatch Repair Deficiency in Endometrial Cancer. *JNCI J. Natl. Cancer Inst.* **2021**, *113*, 1212–1220. [CrossRef]
21. Ma, J.; Setton, J.; Lee, N.Y.; Riaz, N.; Powell, S.N. The therapeutic significance of mutational signatures from DNA repair deficiency in cancer. *Nat. Commun.* **2018**, *9*, 3292. [CrossRef]
22. Oaknin, A.; Gilbert, L.; Tinker, A.V.; Brown, J.; Mathews, C.; Press, J.; Sabatier, R.; O'malley, D.M.; Samouelian, V.; Boni, V.; et al. Safety and antitumor activity of dostarlimab in patients with advanced or recurrent DNA mismatch repair deficient/microsatellite instability-high (dMMR/MSI-H) or proficient/stable (MMRp/MSS) endometrial cancer: Interim results from GARNET—A phase I, single-arm study. *J. Immunother. Cancer* **2022**, *10*, e003777. [CrossRef] [PubMed]
23. Kasherman, L.; Ahrari, S.; Lheureux, S. Dostarlimab in the treatment of recurrent or primary advanced endometrial cancer. *Future Oncol.* **2021**, *17*, 877–892. [CrossRef]
24. Luchini, C.; Bibeau, F.; Ligtenberg, M.J.L.; Singh, N.; Nottegar, A.; Bosse, T.; Miller, R.; Riaz, N.; Douillard, J.-Y.; Andre, F.; 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.* **2019**, *30*, 1232–1243. [CrossRef]
25. Boland, C.R.; Thibodeau, S.N.; Hamilton, S.R.; Sidransky, D.; Eshleman, J.R.; Burt, R.W.; Meltzer, S.J.; Rodriguez-Bigas, M.A.; Fodde, R.; Ranzani, G.N.; et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.* **1998**, *58*, 5248–5257.
26. Wu, X.; Snir, O.; Rottmann, D.; Wong, S.; Buza, N.; Hui, P. Minimal microsatellite shift in microsatellite instability high endometrial cancer: A significant pitfall in diagnostic interpretation. *Mod. Pathol.* **2019**, *32*, 650–658. [CrossRef] [PubMed]
27. Lin, J.H.; Chen, S.; Pallavajjala, A.; Guedes, L.B.; Lotan, T.L.; Bacher, J.W.; Eshleman, J.R. Validation of Long Mononucleotide Repeat Markers for Detection of Microsatellite Instability. *J. Mol. Diagn.* **2021**, *24*, 144–157. [CrossRef]
28. Graham, R.P.; Kerr, S.E.; Butz, M.L.; Thibodeau, S.N.; Halling, K.C.; Smyrk, T.C.; Dina, M.A.; Waugh, V.M.; Rumilla, K.M. Heterogenous MSH6 Loss Is a Result of Microsatellite Instability Within MSH6 and Occurs in Sporadic and Hereditary Colorectal and Endometrial Carcinomas. *Am. J. Surg. Pathol.* **2015**, *39*, 1370–1376. [CrossRef]
29. Hissong, E.; Crowe, E.P.; Yantiss, R.K.; Chen, Y.-T. Assessing colorectal cancer mismatch repair status in the modern era: A survey of current practices and re-evaluation of the role of microsatellite instability testing. *Mod. Pathol.* **2018**, *31*, 1756–1766. [CrossRef] [PubMed]
30. Baudhuin, L.M.; Burgart, L.J.; Leontovich, O.; Thibodeau, S.N. Use of microsatellite instability and immunohistochemistry testing for the identification of individuals at risk for lynch syndrome. *Fam. Cancer* **2005**, *4*, 255–265. [CrossRef]

31. Malapelle, U.; Parente, P.; Pepe, F.; De Luca, C.; Cerino, P.; Covelli, C.; Balestrieri, M.; Russo, G.; Bonfitto, A.; Pisapia, P.; et al. Impact of Pre-Analytical Factors on MSI Test Accuracy in Mucinous Colorectal Adenocarcinoma: A Multi-Assay Concordance Study. *Cells* **2020**, *9*, 2019. [CrossRef]
32. Stelloo, E.; Jansen, A.M.L.; Osse, E.M.; Nout, R.A.; Creutzberg, C.L.; Ruano, D.; Church, D.N.; Morreau, H.; Smit, V.; van Wezel, T.; et al. Practical guidance for mismatch repair-deficiency testing in endometrial cancer. *Ann. Oncol.* **2017**, *28*, 96–102. [CrossRef] [PubMed]
33. Malapelle, U.; Parente, P.; Pepe, F.; De Luca, C.; Pisapia, P.; Sgariglia, R.; Nacchio, M.; Gragnano, G.; Russo, G.; Conticelli, F.; et al. Evaluation of Micro Satellite Instability and Mismatch Repair Status in Different Solid Tumors: A Multicenter Analysis in a Real World Setting. *Cells* **2021**, *10*, 1878. [CrossRef] [PubMed]
34. Lee, M.; Chun, S.-M.; Sung, C.O.; Kim, S.Y.; Kim, T.W.; Jang, S.J.; Kim, J. Clinical Utility of a Fully Automated Microsatellite Instability Test with Minimal Hands-on Time. *J. Pathol. Transl. Med.* **2019**, *53*, 386–392. [CrossRef] [PubMed]
35. Momeni-Boroujeni, A.; Salazar, P.; Zheng, T.; Mensah, N.; Rijo, I.; Dogan, S.; Yao, J.; Moung, C.; Vanderbilt, C.; Benhamida, J.; et al. Rapid EGFR Mutation Detection Using the Idylla Platform: Single-Institution Experience of 1200 Cases Analyzed by an In-House Developed Pipeline and Comparison with Concurrent Next-Generation Sequencing Results. *J. Mol. Diagn.* **2020**, *23*, 310–322. [CrossRef]
36. Safdar, S.; Lammertyn, J.; Spasic, D. RNA-Cleaving NAzymes: The Next Big Thing in Biosensing? *Trends Biotechnol.* **2020**, *38*, 1343–1359. [CrossRef] [PubMed]
37. Libera, L.; Sahnane, N.; Pepe, F.; Pisapia, P.; De Luca, C.; Russo, G.; Parente, P.; Covelli, C.; Chiaravalli, A.M.; Sessa, F.; et al. Critical aspects of microsatellite instability testing in endometrial cancer: A comparison study. *Hum. Pathol.* **2022**, *128*, 134–140. [CrossRef]
38. Umar, A.; Boland, C.R.; Terdiman, J.P.; Syngal, S.; de la Chapelle, A.; Rüschoff, J.; Fishel, R.; Lindor, N.M.; Burgart, L.J.; Hamelin, R.; et al. Revised Bethesda Guidelines for Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome) and Microsatellite Instability. *J. Natl. Cancer Inst.* **2004**, *96*, 261–268. [CrossRef] [PubMed]
39. Ukkola, I.; Nummela, P.; Pasanen, A.; Kero, M.; Lepistö, A.; Kytölä, S.; Bützow, R.; Ristimäki, A. Detection of microsatellite instability with Idylla MSI assay in colorectal and endometrial cancer. *Virchows Arch.* **2021**, *479*, 471–479. [CrossRef]
40. Kullmann, F.; Strissel, P.L.; Strick, R.; Stoehr, R.; Eckstein, M.; Bertz, S.; Wullich, B.; Sikic, D.; Wach, S.; Taubert, H.; et al. Frequency of microsatellite instability (MSI) in upper tract urothelial carcinoma: Comparison of the Bethesda panel and the Idylla MSI assay in a consecutively collected, multi-institutional cohort. *J. Clin. Pathol.* **2023**, *76*, 126–132. [CrossRef]
41. Sutter, C.; Gebert, J.; Bischoff, P.; Herfarth, C.; von Knebel Doeberitz, M. Molecular screening of potential HNPCC patients using a multiplex microsatellite PCR system. *Mol. Cell. Probes* **1999**, *13*, 157–165. [CrossRef]
42. Bacher, J.W.; Flanagan, L.A.; Smalley, R.L.; Nassif, N.A.; Burgart, L.J.; Halberg, R.B.; Megid, W.M.A.; Thibodeau, S.N. Development of a Fluorescent Multiplex Assay for Detection of MSI-High Tumors. *Dis. Markers* **2004**, *20*, 237–250. [CrossRef] [PubMed]
43. Bacher, J.W.; Sievers, C.K.; Albrecht, D.M.; Grimes, I.C.; Weiss, J.M.; Matkowskyj, K.A.; Agni, R.M.; Vyazunova, I.; Clipson, L.; Storts, D.R.; et al. Improved Detection of Microsatellite Instability in Early Colorectal Lesions. *PLoS ONE* **2015**, *10*, e0132727. [CrossRef] [PubMed]
44. Vrenna, F.; Nocita, A.; Alberto, G.; Patarino, R.; Tallarigo, F. Morphological and Molecular Genetic Retrospective Analysis of KRAS, NRAS, BRAF and MSI (Microsatellite Instability) Pattern in Colorectal Cancer. *Gastroenterol. Med. Res.* **2023**, *7*, 648–657. [CrossRef]
45. De Summa, S.; Danza, K.; Pilato, B.; Matera, G.; Fasano, R.; Calabrese, A.; Lacalamita, R.; Silvestris, N.; Tommasi, S.; Argentiero, A.; et al. A Promising Role of TGF-β Pathway in Response to Regorafenib in Metastatic Colorectal Cancer: A Case Report. *Medicina* **2021**, *57*, 1241. [CrossRef] [PubMed]
46. Grespi, V.; Caprera, C.; Ricciolini, C.; Bicchi, I.; Muzi, G.; Corsi, M.; Ascani, S.; Vescovi, A.L.; Gelati, M. Human neural stem cells drug product: Microsatellite instability analysis. *PLoS ONE* **2022**, *17*, e0273679. [CrossRef] [PubMed]
47. Casas-Arozamena, C.; Cortegoso, A.; Piñeiro-Perez, R.; Abalo, A.; Arias, E.; Sampayo, V.; Vilar, A.; Bouso, M.; Diaz, E.; Moreno-Bueno, G.; et al. Improving the Management of Endometrial Cancer Patients through the Use of Liquid Biopsy Analyses: A Case Report. *Int. J. Mol. Sci.* **2022**, *23*, 8539. [CrossRef] [PubMed]
48. Casas-Arozamena, C.; Moiola, C.P.; Vilar, A.; Bouso, M.; Cueva, J.; Cabrera, S.; Sampayo, V.; Arias, E.; Abalo, A.; García, Á.; et al. Noninvasive detection of microsatellite instability in patients with endometrial cancer. *Int. J. Cancer* **2023**, *152*, 2206–2217. [CrossRef] [PubMed]
49. Gilson, P.; Levy, J.; Rouyer, M.; Demange, J.; Husson, M.; Bonnet, C.; Salleron, J.; Leroux, A.; Merlin, J.-L.; Harlé, A. Evaluation of 3 molecular-based assays for microsatellite instability detection in formalin-fixed tissues of patients with endometrial and colorectal cancers. *Sci. Rep.* **2020**, *10*, 16386. [CrossRef] [PubMed]
50. Shia, J.; Tang, L.H.; Vakiani, E.; Guillem, J.G.; Stadler, Z.K.; Soslow, R.A.; Katabi, N.; Weiser, M.R.; Paty, P.B.; Temple, L.K.; et al. Immunohistochemistry as first-line screening for detecting colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome: A 2-antibody panel may be as predictive as a 4-antibody panel. *Am. J. Surg. Pathol.* **2009**, *33*, 1639–1645. [CrossRef] [PubMed]
51. Mirza, M.R.; Chase, D.M.; Slomovitz, B.M.; dePont Christensen, R.; Novák, Z.; Black, D.; Gilbert, L.; Sharma, S.; Valabrega, G.; Landrum, L.M.; et al. Dostarlimab for Primary Advanced or Recurrent Endometrial Cancer. *N. Engl. J. Med.* **2023**, *388*, 2145–2158. [CrossRef]

52. Zannoni, G.F.; Santoro, A.; D'alessandris, N.; Scaglione, G.; Inzani, F.; Angelico, G.; Bragantini, E.; Piermattei, A.; Cianfrini, F.; Bisaro, B.; et al. Biomarker characterization in endometrial cancer in Italy: First survey data analysis. *Pathologica* **2022**, *114*, 189–198. [CrossRef] [PubMed]
53. Raffone, A.; Travaglino, A.; Cerbone, M.; Gencarelli, A.; Mollo, A.; Insabato, L.; Zullo, F. Diagnostic Accuracy of Immunohistochemistry for Mismatch Repair Proteins as Surrogate of Microsatellite Instability Molecular Testing in Endometrial Cancer. *Pathol. Oncol. Res.* **2020**, *26*, 1417–1427. [CrossRef] [PubMed]
54. Cicek, M.S.; Lindor, N.M.; Gallinger, S.; Bapat, B.; Hopper, J.L.; Jenkins, M.A.; Young, J.; Buchanan, D.; Walsh, M.D.; Le Marchand, L.; et al. Quality Assessment and Correlation of Microsatellite Instability and Immunohistochemical Markers among Population- and Clinic-Based Colorectal Tumors: Results from the Colon Cancer Family Registry. *J. Mol. Diagn.* **2011**, *13*, 271–281. [CrossRef] [PubMed]
55. Loughrey, M.B.; McGrath, J.; Coleman, H.G.; Bankhead, P.; Maxwell, P.; McGready, C.; Bingham, V.; Humphries, M.P.; Craig, S.G.; McQuaid, S.; et al. Identifying mismatch repair-deficient colon cancer: Near-perfect concordance between immunohistochemistry and microsatellite instability testing in a large, population-based series. *Histopathology* **2020**, *78*, 401–413. [CrossRef] [PubMed]
56. Bai, H.; Wang, R.; Cheng, W.; Shen, Y.; Li, H.; Xia, W.; Ding, Z.; Zhang, Y. Evaluation of Concordance Between Deficient Mismatch Repair and Microsatellite Instability Testing and Their Association with Clinicopathological Features in Colorectal Cancer. *Cancer Manag. Res.* **2020**, *12*, 2863–2873. [CrossRef] [PubMed]
57. Overbeek, L.I.; Ligtenberg, M.J.; Willems, R.W.; Hermens, R.P.; Blokx, W.A.; Dubois, S.V.; van der Linden, H.; Meijer, J.W.; Mlynek-Kersjes, M.L.; Hoogerbrugge, N.; et al. Interpretation of immunohistochemistry for mismatch repair proteins is only reliable in a specialized setting. *Am. J. Surg. Pathol.* **2008**, *32*, 1246–1251. [CrossRef] [PubMed]
58. Streel, S.; Salmon, A.; Dheur, A.; Bours, V.; Leroi, N.; Habran, L.; Delbecque, K.; Goffin, F.; Pleyers, C.; Kakkos, A.; et al. Diagnostic Performance of Immunohistochemistry Compared to Molecular Techniques for Microsatellite Instability and p53 Mutation Detection in Endometrial Cancer. *Int. J. Mol. Sci.* **2023**, *24*, 4866. [CrossRef] [PubMed]
59. Cohen, R.; Hain, E.; Buhard, O.; Guilloux, A.; Bardier, A.; Kaci, R.; Bertheau, P.; Renaud, F.; Bibeau, F.; Fléjou, J.-F.; et al. Association of Primary Resistance to Immune Checkpoint Inhibitors in Metastatic Colorectal Cancer With Misdiagnosis of Microsatellite Instability or Mismatch Repair Deficiency Status. *JAMA Oncol.* **2019**, *5*, 551–555. [CrossRef] [PubMed]
60. Wu, S.; Liu, X.; Wang, J.; Zhou, W.; Guan, M.; Liu, Y.; Pang, J.; Lu, T.; Zhou, L.; Shi, X.; et al. DNA Mismatch Repair Deficiency Detection in Colorectal Cancer by a New Microsatellite Instability Analysis System. *Interdiscip. Sci.* **2020**, *12*, 145–154. [CrossRef]
61. Bacher, J.W.; Udho, E.B.; Strauss, E.E.; Vyazunova, I.; Gallinger, S.; Buchanan, D.D.; Pai, R.K.; Templeton, A.S.; Storts, D.R.; Eshleman, J.R.; et al. A Highly Sensitive Pan-Cancer Test for Microsatellite Instability. *J. Mol. Diagn.* **2023**, *25*, 806–826. [CrossRef]
62. Parente, P.; Malapelle, U.; Angerilli, V.; Balistreri, M.; Lonardi, S.; Pucciarelli, S.; De Luca, C.; Pepe, F.; Russo, G.; Vigliar, E.; et al. MMR profile and microsatellite instability status in colorectal mucinous adenocarcinoma with synchronous metastasis: A new clue for the clinical practice. *J. Clin. Pathol.* **2022**, *76*, 492–496. [CrossRef] [PubMed]
63. Haruma, T.; Nagasaka, T.; Nakamura, K.; Haraga, J.; Nyuya, A.; Nishida, T.; Goel, A.; Masuyama, H.; Hiramatsu, Y. Clinical impact of endometrial cancer stratified by genetic mutational profiles, POLE mutation, and microsatellite instability. *PLoS ONE* **2018**, *13*, e0195655. [CrossRef] [PubMed]
64. Pécriaux, A.; Favre, L.; Calderaro, J.; Charpy, C.; Derman, J.; Pujals, A. Detection of microsatellite instability in a panel of solid tumours with the Idylla MSI Test using extracted DNA. *J. Clin. Pathol.* **2020**, *74*, 36–42. [CrossRef]
65. Orellana, T.J.; Kim, H.; Beriwal, S.; Taylor, S.E.; Smith, K.J.; Lesnock, J.L. Cost-effectiveness analysis of tumor molecular testing in stage III endometrial cancer. *Gynecol. Oncol.* **2023**, *173*, 81–87. [CrossRef] [PubMed]
66. Vikas, P.; Messersmith, H.; Compton, C.; Sholl, L.; Broaddus, R.R.; Davis, A.; Estevez-Diz, M.; Garje, R.; Konstantinopoulos, P.A.; Leiser, A.; et al. Mismatch Repair and Microsatellite Instability Testing for Immune Checkpoint Inhibitor Therapy: ASCO Endorsement of College of American Pathologists Guideline. *J. Clin. Oncol.* **2023**, *41*, 1943–1948. [CrossRef]
67. Bartels, S.; Grote, I.; Wagner, M.; Boog, J.; Schipper, E.; Reineke-Plaass, T.; Kreipe, H.; Lehmann, U. Concordance in detection of microsatellite instability by PCR and NGS in routinely processed tumor specimens of several cancer types. *Cancer Med.* **2023**, *12*, 16707–16715. [CrossRef]
68. Evrard, C.; Cortes, U.; Ndiaye, B.; Bonnemort, J.; Martel, M.; Aguillon, R.; Tougeron, D.; Karayan-Tapon, L. An Innovative and Accurate Next-Generation Sequencing-Based Microsatellite Instability Detection Method for Colorectal and Endometrial Tumors. *Mod. Pathol.* **2024**, *104*, 100297. [CrossRef]