



Original research

# Extent of investigation and management of cases of 'unexplained' mismatch repair deficiency (u-dMMR): a UK Cancer Genetics Group consensus

Terri Patricia McVeigh <sup>1,2</sup>, Kevin J Monahan,<sup>3,4</sup> Joseph Christopher,<sup>5,6</sup> Nick West,<sup>7,8</sup> Malcolm Scott,<sup>9</sup> Jennie Murray,<sup>10</sup> Helen Hanson <sup>11,12</sup> UKCGG dMMR Consensus Meeting Attendees

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For numbered affiliations see end of article.

## Correspondence to

Dr Terri Patricia McVeigh, Cancer Genetics Unit, Royal Marsden Hospital NHS Trust, London SW3 6JJ, UK; [terri.mcveigh@nhs.net](mailto:terri.mcveigh@nhs.net)  
Dr Helen Hanson;  
[h.hanson@exeter.ac.uk](mailto:h.hanson@exeter.ac.uk)

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## ABSTRACT

**Background** Mismatch repair deficiency (dMMR) is a characteristic feature of cancers linked to Lynch syndrome. However, in most cases, it results from sporadic somatic events rather than hereditary factors. The term 'Lynch-like syndrome' (LLS) has been used to guide colorectal cancer surveillance for relatives of individuals with a dMMR tumour when somatic and germline genomic testing is uninformative. As the assessment of mismatch repair through immunohistochemistry and/or microsatellite instability is increasingly applied across various tumour types for treatment planning, dMMR is increasingly detected in tumours where suspicion of hereditary aetiology is low. Our objective was to establish current practices and develop national guidance for investigating, and managing relatives of, patients with cancers demonstrating unexplained dMMR.

**Methods** This was achieved through a virtual consensus meeting involving key stakeholders from the UK, through premeeting surveys, structured discussions and in-meeting polling to formulate best practice guidance.

**Results** We identified variability in the availability of diagnostic technologies across specialist centres. It was agreed that equitable access to baseline testing is required, acknowledging the need for a pragmatic approach to investigating dMMR cancers not traditionally associated with Lynch syndrome. Factors such as family history, age, tumour type, protein loss pattern and extent of the investigation were deemed crucial in guiding family management. The term 'unexplained dMMR' was recommended over LLS.

**Conclusion** Decisions regarding investigations and future cancer risk management in patients and relatives should be nuanced, considering factors like clinical suspicion of hereditary predisposition to allocate limited resources efficiently and avoid unnecessary investigations in low-suspicion families.

## INTRODUCTION

Lynch syndrome is defined by the identification of constitutional pathogenic variants in four mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*). Although functional MMR deficiency (dMMR) is a hallmark of cancers associated with Lynch syndrome, dMMR may also be a feature of

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Mismatch repair deficiency (dMMR) may be due to an underlying heritable variant impacting mismatch repair gene function or may be due to a sporadic, somatic aetiology. Even in cases where germline and somatic testing is undertaken, dMMR in a minority of cases remains unexplained. The investigative pathway and clinical management of patients with tumours demonstrating unexplained dMMR, and their relatives, pose challenges.

## WHAT THIS STUDY ADDS

⇒ Following a national, multidisciplinary consensus meeting, we have outlined a pragmatic approach to investigation of patients with dMMR cancers, guided by clinical suspicion of hereditary predisposition as well as considering available resources in a publicly funded health system.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ We have identified a number of areas where consensus could not be achieved, which warrant further research and long-term prospective data collection and interrogation. Further data are necessary to elucidate the underlying aetiology of dMMR in tumours not classically associated with Lynch syndrome, but undertaking necessary investigations in the National Health Service (NHS) will require further investment in existing services, as well as addressing inequities in access to key technologies.

sporadic cancers as a consequence of somatic aberrations impacting MMR gene function.<sup>1</sup> dMMR, as evidenced by abnormal expression of one or more MMR proteins, and/or microsatellite instability (MSI) is most commonly detected in colorectal (12%–15%) and endometrial (20%–30%) cancers but can be a feature of multiple other cancer types, including upper gastrointestinal<sup>2</sup> (~10%), ovarian (~10%), hepatobiliary (10%) and adrenocortical cancers.<sup>3,4</sup> Given that dMMR cancers typically demonstrate favourable response to immune



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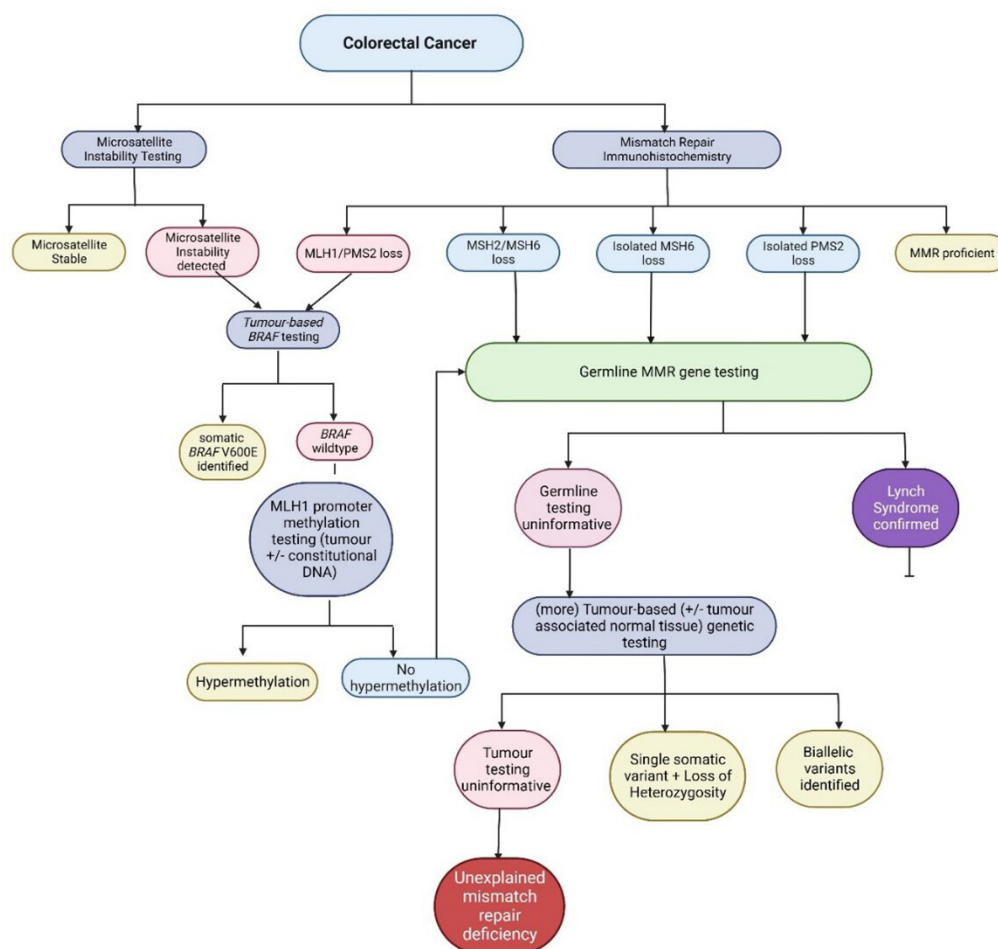
checkpoint inhibitors (ICIs),<sup>5</sup> the expanding availability of such therapeutic agents has fuelled increasing testing for, and detection of, dMMR in a host of cancer types, including common and rare Lynch syndrome-associated malignancies, as well as cancers not usually considered part of the Lynch syndrome phenotypic spectrum.<sup>6</sup>

The majority of dMMR detected in colorectal and endometrial cancers is attributable to sporadic somatic hypermethylation of the *MLH1* promoter,<sup>7,8</sup> and, usually, detection of hypermethylation of the *MLH1* promoter in tumour-derived DNA (or, in colorectal cancer, detection of a pathogenic somatic *BRAF* variant as a surrogate marker of same) will obviate the need for constitutional genetic testing.<sup>9</sup> Testing for constitutional hypermethylation of the *MLH1* promoter, if not undertaken at the time of initial tumour-based analysis, and/or constitutional MMR gene testing may still be warranted if clinical suspicion for Lynch syndrome, based on patient age and/or family history, persists.<sup>10</sup>

At present, in the UK, constitutional and tumour-based genetic testing is undertaken, for the most part, in an unsynchronised, unpaired manner, driven, in part, by increasing awareness of Lynch syndrome as an underlying aetiology for a small but significant proportion of cancer risk but also by increasing application of and access to ICIs. The National Institute for Health and Care Excellence (NICE) has published guidance for assessment of MMR in endometrial and colorectal cancers, with investigative

pathway guided by tumour type and, where available, pattern of MMR protein loss (figure 1).<sup>11,12</sup> Detection of a pathogenic activating *BRAF* variant in colorectal cancer demonstrating loss of *MLH1* (usually with concomitant loss of *PMS2*) can be used as a surrogate marker of *MLH1* promoter hypermethylation, but 18%–20% of cancers demonstrating *MLH1* promoter hypermethylation will not harbour a detectable *BRAF* variant.<sup>9,13</sup> *BRAF* variants are rare in endometrial cancers, such that *BRAF* testing is not usually informative for such cancers.<sup>14</sup> The utility of such testing in other extracolonic cancers is uncertain, as is the contribution of *MLH1* promoter hypermethylation to dMMR in other tumour types.

For patients with cancers demonstrating combined *MLH1*/*PMS2* deficiency and/or microsatellite instability where *MLH1* promoter hypermethylation is not detected, or for patients with cancers demonstrating isolated loss of *PMS2* or *MSH6*, or combined loss of *MSH2* and *MSH6*, or where clinical suspicion for Lynch syndrome persists based on age and/or family history, constitutional MMR gene testing is indicated.<sup>11,12</sup> Type and extent of constitutional testing should be guided by the pattern of protein loss and the a priori suspicion that a constitutional pathogenic variant explains the patient phenotype and in line with criteria outlined in the NHS England Genomic Testing Directory<sup>15</sup> or equivalent in Wales, Northern Ireland and Scotland.<sup>16</sup> In patients with tumours demonstrating isolated *PMS2* deficiency, testing should include long-range PCR of *PMS2* (or



**Figure 1** Pathway for investigation of mismatch repair (MMR) and/or microsatellite instability in colorectal cancers. Decisions about the extent of investigation along this pathway are guided by clinical suspicion and the availability of relevant investigations. If investigation is stopped following germline MMR gene testing, the proportion of unexplained MMR deficiency will be higher.

equivalent assessment of region impacted by pseudogene interference), while for tumours demonstrating loss of MSH2 and/or MSH6, testing should include dosage analysis of 3' end of *EPCAM*, and testing for the recurrent paracentric inversion of exons 1–7 in *MSH2*.<sup>17</sup> Where constitutional testing is uninformative, testing on tumour-derived DNA ('somatic testing') may then be undertaken to determine if an underlying somatic aetiology accounts for dMMR.<sup>18</sup> In those cases where biallelic somatic events (biallelic variants or a single variant with evidence of loss of heterozygosity) impacting an MMR gene are not identified, the dMMR is classified as 'unexplained' (henceforth abbreviated as u-dMMR), and, historically, affected individuals were said to have 'Lynch-like syndrome' (LLS). The proportion of cases deemed 'unexplained' is influenced by the extent of investigation—after somatic *BRAF* sequencing and *MLH1* promoter hypermethylation and, where indicated, constitutional MMR gene testing is undertaken, approximately 4.24% of colorectal cancers have u-dMMR; when somatic MMR analysis is undertaken, this reduces to 0.61%.<sup>19</sup>

Possible explanations for u-dMMR are undetectable somatic or constitutional pathogenic variants.<sup>20</sup> Other explanations may include inaccuracies in the tests performed. For example, the performance of immunohistochemistry (IHC) is dependent on platform (including antibodies) and interpretation of results is observer-dependent and may be influenced by tumour fixation. Therefore, repeating an apparently abnormal IHC result may be useful as a first step in cases where an aetiology cannot be identified, particularly if IHC results are discordant with MSI results, and if clinical suspicion of Lynch syndrome is low.<sup>21 22</sup> Testing an alternative well-fixed specimen, for example, diagnostic biopsy may be helpful to avoid the reduction/loss of staining noted with poorly fixed resection specimens. Consideration should also be given to the possibility of *BRAF* variants and/or *MLH1* hypermethylation being missed by testing a sample with inadequate tumour cellularity.<sup>23</sup>

Published recommendations for bowel screening in relatives of affected probands deemed to have LLS are equivalent to that offered to patients with Lynch syndrome.<sup>24</sup> However, while a proportion may be due to an unidentified heritable event, where clinical suspicion of a hereditary predisposition is low, it is more likely that dMMR will be due to an unidentified somatic aetiology.<sup>25 26</sup> This is evidenced by the fact that relatives of probands with LLS have a lower cancer risk than in those families with molecularly confirmed Lynch syndrome, although higher than in those families with sporadic colorectal cancer.<sup>27</sup>

In a publicly funded health system, it could be argued that it is most appropriate that limited resources are best focused on detection of rare, atypical mechanisms of heritable dMMR<sup>20</sup> and screening of relatives of probands where suspicion of heritable aetiology remains high after uninformative investigations. However, conversely, as screening recommendations can be 'downgraded' once an aetiology for MMR is established, it could be argued that further investigations could be warranted where an explanation for MMR deficiency is not immediately obvious but where clinical suspicion of constitutional mechanism is low.

### National multidisciplinary team meeting

Every two months, a virtual multidisciplinary meeting involving clinical geneticists, genetic counsellors and other healthcare professionals working in Cancer Genetics convenes as part of a national collaborative initiative between the Cancer Research UK-funded CanGene-CanVar Programme and the UK Cancer Genetics Group (UKCGG), a constituent group of the British

**Table 1** Cases discussed at UKCGG/CanGene-CanVar Cancer Genetics MDT

Summary: Clinical scenarios	Consensus
1. Young patient (age 40–50 years) with MMR-deficient endometrial cancer from an Amsterdam-positive family history (unconfirmed). Germline genetic testing uninformative. Single <i>MSH2</i> somatic variant detected.	Manage as Lynch-like unless further information obtained
2. Deceased proband with MMR-deficient colorectal cancer (isolated loss of <i>PMS2</i> ) at 40–50 years. Molecular testing on tissue-derived DNA identified <i>PMS2</i> pathogenic variant—origin (germline/somatic) could not be determined based on available tissue.	Offer germline testing to FDRs for <i>PMS2</i> variant identified in tumour
3. MMR-deficient (loss of <i>MSH2</i> and <i>MSH6</i> ) endometrial cancer at 50–60 years, keratoacanthoma at 50–60 years. Constitutional testing uninformative. Somatic testing identified two somatic <i>MSH2</i> variants at low VAF. Cousin with young onset endometrial and rectal cancer also MMR-deficient (loss of <i>MSH2</i> and <i>MSH6</i> ). Somatic testing failed. Amsterdam-positive family history.	Manage as Lynch-like unless further information obtained
4. Man with MMR-deficient (loss of <i>MLH1</i> and <i>PMS2</i> ) colorectal at 30–40 years. Mother hysterectomy in late 30s. Brother polyps at 40–50 years. Uninformative constitutional testing.	Manage as Lynch-like unless further information obtained
5. MMR-deficient (isolated loss <i>PMS2</i> ) pancreatic cancer in a male aged 70–80 years; constitutional testing uninformative, testing on tumour-derived DNA unsuccessful.	Colonoscopic screening based on family history
6. MMR-deficient (loss of <i>MLH1</i> and <i>PMS2</i> ) colorectal cancer in 60–70 year old male. Mother ovarian cancer in 80s. No <i>MLH1</i> promoter hypermethylation or constitutional MMR gene variants identified. Single somatic <i>MLH1</i> variant identified.	National bowel screening
FDR, first-degree relative; MDT, multidisciplinary team; MMR, mismatch repair; UKCGG, UK Cancer Genetics Group; VAF, Variant Allele Frequency.	

Society of Genomic Medicine.<sup>28</sup> Each meeting has a specific theme, and clinicians are invited to submit challenging cases aligned with that theme. The session in November 2022 was on the theme of u-dMMR. A number of cases were presented (table 1), all of whom were patients with dMMR cancers where aetiology was not established. The audience was asked to comment on bowel screening recommendations based on clinical features (eg, age of cancer diagnoses, cancer type, family history and results of tumour and constitutional testing). Recommendations ranged from population bowel cancer screening for relatives to Lynch-equivalent bowel screening.

During the meeting, consensus was easily achieved for bowel screening recommendations for each case, although this was only informally recorded given the nature of the meeting. From the discussions, it was noted that most centres consider tumour type, age and family history when determining extent of testing to establish aetiology of dMMR and screening recommendations for relatives. However, it was noted that regional variability in access to certain technologies such as long-range PCR for *PMS2* and loss of heterozygosity analysis was a limitation.

In cases where constitutional MMR testing is uninformative and clinical suspicion of Lynch syndrome low, there was discussion about whether the cumulative human resources and cost required in undertaking additional tumour-based testing to try to determine aetiology for dMMR is unacceptably high when considering diagnostic yield. It was questioned whether this would be cost-effective considering numbers needed to test/screen to prevent one cancer-related death. It was suggested that tumour-based testing to establish somatic aetiology of dMMR might be most appropriate in those cases where clinical suspicion of a heritable predisposition is at least moderate, in order to determine the most appropriate familial bowel screening

recommendations; and ensure limited resources and highest intensity screening are directed to those families where suspicion for Lynch syndrome remains high. To more fully address these issues, the UKCGG convened a national consensus meeting on 25 April 2023.

### National consensus meeting preparation and format

Key experts representing gastroenterology, pathology, gynaecology and clinical genetics from each genomic medicine service alliance and each of the four countries in the UK were invited to contribute to a national consensus meeting. Participants from the Republic of Ireland were also permitted to attend. In advance of the meeting, an electronic survey was circulated to cancer genetics leads in each regional clinical genetics service, to establish current practices across the UK.

Specifically, attendees were asked to comment on:

- ▶ Their approach to different clinical scenarios regarding colorectal surveillance strategies
- ▶ The extent to which testing was completed according to the algorithm in figure 1, according to patients of different ages with typical versus atypical Lynch syndrome spectrum tumours (online supplemental table 1)
- ▶ Access to testing in their centres (online supplemental figure 1), and what testing they felt *should* be available.
- ▶ The factors that should be considered in determining the likelihood of a heritable cause of dMMR cancer, for example, age and tumour type.
- ▶ When molecular testing could be considered completed in order to make clinical recommendations (ie, was 'exhaustive' testing required as standard or could a more pragmatic approach be taken?)

Key expert speakers were invited to give an overview of current evidence on prevalence of, and approaches to testing for, dMMR across a host of tumour types. The agenda of the meeting is outlined in appendix 1. These talks provided the multidisciplinary audience with relevant overview of evidence on which to base their responses to proposed statements. Recordings of these talks and copies of speakers' slides are available

to download (<https://www.ukcgg.org/information-education/ukcgg-consensus-meetings/>). In-meeting polls were then run to achieve consensus on a number of statements generated based on thematic analysis of survey results. In line with standard Delphi processes, consensus was deemed to be achieved when at least 50 responses (approximately half of respondents to which relevant statements applied) were recorded, and when at least 80% of responses were 'agree/strongly agree' with the proposed statement.<sup>29</sup> Open discussion was encouraged where minimum number of responses or consensus was not achieved, or if audience members indicated strong feelings in contravention of the proposed statement. This approach has been successfully implemented for several other UKCGG-led national consensus meetings to generate clinical guidelines.<sup>30–34</sup>

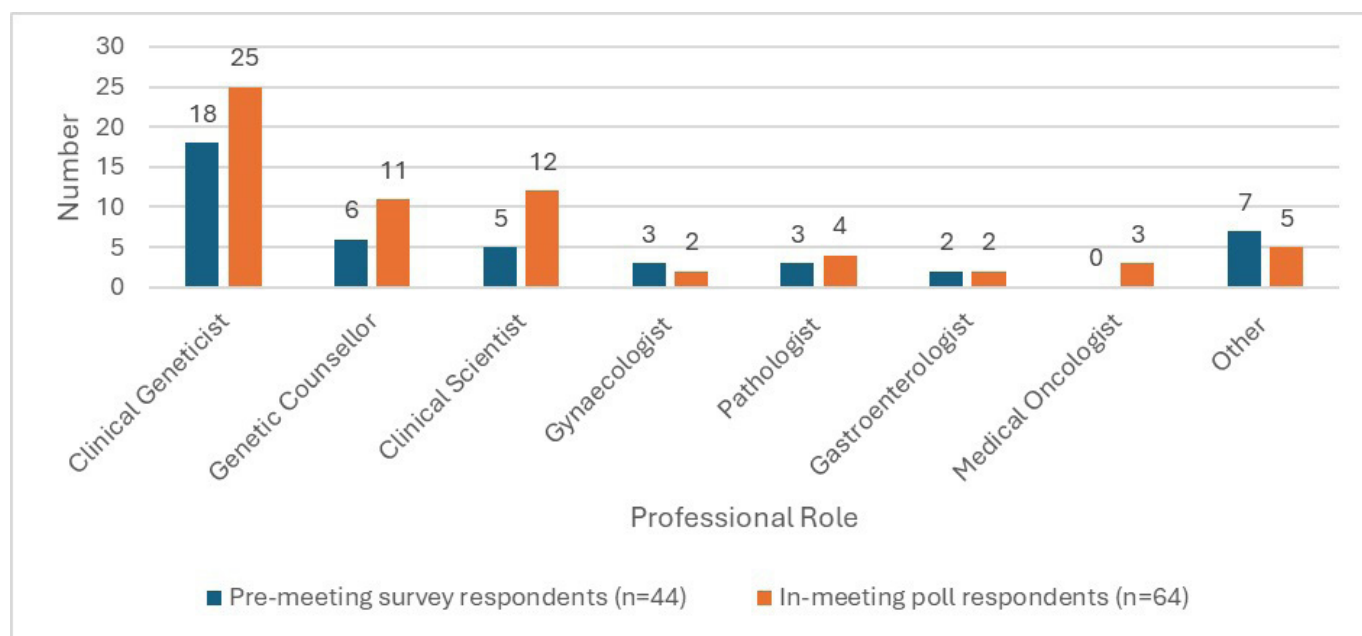
## RESULTS

### Premeeting survey

The premeeting survey was completed by 44 respondents. A response to a question was considered 'valid' if not left blank. The largest single professional group of respondents were clinical geneticists (n=18) (figure 2).

In most centres, MMR IHC was the preferred method of assessment of MMR for colorectal cancer and endometrial cancer. Most respondents indicated that reflex testing of MMR was being undertaken on all colorectal (35/36 valid responses) and endometrial cancers (33/36 valid responses), but some respondents indicated that such testing was also reflexively undertaken across a host of other tumour types (online supplemental table 1).

Most respondents indicated that they had routine access to tumour-based *BRAF* and *MLH1* promoter hypermethylation testing, with a slightly lower proportion of respondents indicating routine access to constitutional or tumour-based MMR gene sequencing. Fewer than half of respondents indicated routine access to long-range PCR testing of *PMS2* (13/37 valid responses) or loss of heterozygosity analysis on tumour-derived DNA (15/37 valid responses) (online supplemental figure 1).



**Figure 2** Professional roles of respondents to premeeting survey and in-meeting polls. Other: clinical nurse specialist, colorectal surgeon, oncologist, patient representative, not specified.



**Table 2** Statements on which consensus was achieved

Statement	Responses (n)	Proportion of respondents indicating 'agree/ strongly agree' or 'yes' (%)
The abbreviations that should be used to define MMR proficient and MMR deficient should be 'pMMR' and 'dMMR' as per the IARC 'Blue Book' volume on genetic tumour syndromes.	58	96
The term unexplained dMMR should be used instead of the term 'Lynch-like syndrome'.	60	88
Long-range PCR analysis of PMS2 should routinely be included as part of constitutional testing of PMS2 when cancer demonstrates isolated loss of PMS2.	59	85
Loss of heterozygosity analysis should be routinely available when somatic next-generation sequencing of MMR genes is undertaken.	59	95
It is not mandatory to pursue additional investigations for patients with dMMR cancers beyond standard testing* and constitutional genetic testing in situations where prior probability of heritable cause for cancer is low.	55	90
The following factors should be considered in determining probability of heritable cause of dMMR cancer to guide further testing: family history, age, tumour type, pattern of protein loss, extent of investigation.	54	94
It is not mandatory to undertake Lynch-equivalent surveillance for probands or their first-degree relatives in situations where there is unexplained dMMR, particularly where clinical suspicion of a heritable cause is low.	56	96

\*Standard testing: somatic *BRAF* sequencing and/or *MLH1* promoter hypermethylation testing where indicated; constitutional MMR gene testing. IARC, International Agency for Research on Cancer; MMR, mismatch repair.

Variability in extent of testing offered in different clinical scenarios was noted; with some centres offering the same extent of testing in all circumstances, and other services offering no further testing to older patients after uninformative germline results. Similar variability in screening recommendations was noted, with some centres recommending Lynch-equivalent screening for relatives of all probands with unexplained dMMR cancers irrespective of age at diagnosis.

### Consensus meeting attendees

The consensus meeting was attended by 82 participants. Because of the multidisciplinary nature of the audience, not all statements were applicable to each attendee, for which reason complete participation for every poll was not expected. 64 participants participated in at least one poll (figure 2).

### In-meeting polls

#### Statements for which consensus was achieved

There were a number of statements for which consensus was achieved after discussion (table 2).

#### Terminology

It was agreed that the abbreviations that should be used to define MMR proficient and MMR deficient should be pMMR and dMMR, respectively, as per the International Agency for Research on Cancer 'Blue Book' volume on genetic tumour syndromes<sup>35</sup> (responses: 58; agreement: 96%). It was also agreed that the term u-dMMR should be used instead of the term Lynch-like Syndrome (LSS) (responses: 60; agreement: 88%).

#### Extent of molecular testing for dMMR cancer

Participants were asked to comment on the extent of investigation for patients with dMMR cancers beyond 'standard testing' (that specified by NICE guidelines DG27<sup>11</sup>/DG42<sup>12</sup> or the NHSE Genomic Test Directory (or equivalent in devolved nations)). It was agreed that long-range PCR analysis of *PMS2* should routinely be included as part of constitutional genetic testing if isolated loss of *PMS2* was observed in the cancer (responses: 59; agreement: 85%), and that loss of heterozygosity analysis should be routinely available when somatic next-generation sequencing of MMR genes is undertaken on tumour-derived DNA (responses: 59; agreement: 95%). It was also agreed that it should not be considered mandatory to pursue additional

investigations for patients with dMMR cancers beyond standard testing in situations where prior probability of heritable cause for cancer is low (responses: 55; agreement: 90%).

#### Factors affecting clinical interpretation of dMMR results and need for further testing or enhanced colorectal cancer screening

It was agreed by attendees that although a baseline level of testing as outlined in NICE guidelines and genomic test directories was clinically appropriate for evaluation of patients with dMMR cancers, a pragmatic approach could be taken with regard to the extent of testing for dMMR cancers beyond such testing where the index of suspicion of Lynch syndrome was low for example, for dMMR cancers in older patients without a family history of cancer.

In determining the likelihood of heritable cause of dMMR, and therefore to guide further testing, it was agreed that family history, age, tumour type, pattern of protein loss and extent of investigation (including availability of long-range PCR testing of *PMS2*) should be considered (responses: 54; agreement: 94%).

In determining screening recommendations for the proband with dMMR cancer and their first-degree relatives (FDRs), it was agreed that it was not always appropriate to recommend Lynch-equivalent surveillance where clinical suspicion of a heritable cause is low, and that such recommendations should not be considered mandatory (responses: 56; agreement: 96%).

#### Statements where consensus could not be achieved

Despite extensive debate and discussion, there were specific statements for which consensus could not be reached, a variance which reflects uncertainty in testing and clinical interpretation, and difficulty in making general recommendations given the wide variability in clinical scenarios in which dMMR will be encountered (table 3).

#### Extent of molecular testing for dMMR cancer

It was suggested that comprehensive testing of dMMR colorectal and endometrial cancers should include *BRAF* and/or *MLH1* promoter hypermethylation testing, constitutional MMR genetic testing, somatic MMR next-generation sequencing and loss of heterozygosity analysis and that these investigations should be performed to rule out a diagnosis of Lynch syndrome prior to making surveillance recommendations. Consensus could not be reached on this point (responses: 54; agreement:

**Table 3** Statements for which minimum number of responses and/or consensus was not achieved

Statement	Responses (n) (minimum required: 50)	Proportion of respondents indicating 'agree/ strongly agree' or 'yes' (%) (minimum required: 80%)
For all colorectal and endometrial cancers, comprehensive testing ( <i>BRAF/MLH1</i> promoter hypermethylation testing/germline testing/somatic MMR next-generation sequencing and loss of heterozygosity analysis) should be performed to rule out a diagnosis of Lynch syndrome prior to making surveillance recommendations.	54	60
Additional investigations for patients with dMMR cancers beyond standard testing as per DG27 <sup>11</sup> /DG42 <sup>12</sup> and germline genetic testing depend on prior probability of heritable cause for cancer and are not necessary where clinical suspicion is low.	45	60
For proband with dMMR cancer diagnosed >65 years of age without a family history of Lynch syndrome-associated cancers, colorectal surveillance in the proband and their relatives should be guided by the clinical picture and specialist MDT discussion.	24	83
Lynch-equivalent screening (for probands and first-degree relatives) is only appropriate where clinical suspicion of a heritable cause for dMMR is high.	47	75
The extent of testing for unexplained dMMR prior to making surveillance recommendations may be adjusted for non-colorectal/non-endometrial cancers.	7	100
Age and family history should be considered when deciding to proceed to additional testing beyond <i>BRAF/MLH1</i> hypermethylation and germline testing, for colorectal and endometrial cancers to rule out a diagnosis of Lynch syndrome prior to making surveillance recommendations.	13	69
Where minimum number of responses have not been achieved, or where the proportion of respondents indicating "agree"/"strongly agree" is less than 80%, the values are in bold.		

60%), with participants citing logistic issues in accessing relevant samples and certain diagnostic technologies, available capacity and resources within clinical departments and lengthy time for full diagnostic workup as barriers to provision of timely, fully informed screening recommendations. Many clinicians provide interim screening recommendations with a view to reviewing the need for screening if updated information from testing becomes available.

It was suggested that additional investigations for patients with dMMR cancers beyond standard testing should be guided by the prior probability of heritable cause for cancer and is not necessary where clinical suspicion is low. Consensus could not be achieved for this statement (responses: 45; agreement: 60%), with many participants citing that such investigations may be most helpful where clinical suspicion of hereditary predisposition is low, in order to 'downgrade' screening recommendations. Ultimately, after discussion, consensus was reached that the likelihood of heritable predisposition could be considered in guiding further testing, as outlined above and may be best undertaken in a local MDT setting.

#### *Factors affecting clinical interpretation of dMMR results and need for further testing or enhanced colorectal cancer screening*

When attempting to determine the indications for, and extent of additional investigations (beyond germline genetic testing) for patients with dMMR cancers, there was a lack of consensus as to how a prior probability of diagnosing Lynch syndrome should be evaluated and implemented. Discussion around this focused on lack of suitable models to estimate this probability, a minority of participants indicating use of models such as PREMM5 algorithm in clinical practice, however, while this is a useful tool to estimate the probability of Lynch syndrome prior to constitutional testing, it does not currently consider residual likelihood post-testing.<sup>36</sup> Consensus could not be achieved when it was proposed that Lynch equivalent screening be limited to those families where suspicion of Lynch syndrome remained high after uninformative constitutional testing (responses: 47; agreement: 75%), for the same reasons. However, consensus was achieved when the statement was reframed to state that such screening was not mandatory if clinical suspicion for Lynch syndrome

was low, as outlined above. Participants were reluctant to use age at diagnosis in isolation, as a factor to determine further investigation/screening.

There was uncertainty as to whether surveillance recommendations would be modified based on tumour site as well as clinical features, with most participants abstaining from commenting on the proposed statement, although those participants who did respond agreed this would be acceptable (responses: 7; agreement: 100%).

#### **DISCUSSION**

The term LLS refers to an intermediate state of testing, and not a distinct heritable syndrome per se, and has therefore never been clearly defined despite advances in molecular testing and evaluation. Thus, some authors have suggested that the term may cause confusion and is redundant and should not be used.<sup>35 37 38</sup> The consensus in this meeting was that the term 'u-dMMR' more accurately reflected intermediate and/or uncertainty in testing for dMMR cancers.

There remain significant diagnostic and management challenges when considering patients with cancers demonstrating u-dMMR. Although it was agreed by participants that the term LLS was ill-defined and obsolete, this term has been used in clinical guidelines for colorectal cancer screening in relatives of probands with u-dMMR cancers. The redefinition of LLS to 'u-dMMR' by this consensus meeting therefore necessitates a reassessment of existing clinical guidelines<sup>24</sup> to align with our consensus indicating that Lynch syndrome-equivalent surveillance is not always appropriate or necessary in families where one individual is affected by u-dMMR cancer.

Significant discussion was generated regarding the extent of testing required to 'rule out' Lynch syndrome before surveillance recommendations could be made, with participants citing issues related to access to certain technologies and resources required to perform such testing. Many participants also highlighted the different paradigms in arranging tests to 'exclude' a hereditary predisposition, compared with most clinical scenarios where resources are generally focused on diagnosis of heritable mechanisms of disease. The likelihood of Lynch syndrome can be significantly reduced in the majority of patients with dMMR

cancers following constitutional testing, and therefore a pragmatic approach to additional testing and ongoing surveillance for family members may be justifiable in some situations. Limitations of genomic testing not unique to Lynch syndrome but to all inherited conditions are such that heritable risk factors can never be fully excluded, and the considerable resources required to investigate dMMR cancers over and above an uninformative germline test, where the prior probability of Lynch syndrome is low must be considered.

Much discussion and variance in opinion focused on lack of data regarding proportion of non-colorectal/non-endometrial dMMR cancers accounted for by Lynch syndrome. This was clearly an issue when considering statements related to extent of investigation/screening recommendations for probands with non-colorectal/non-endometrial cancers.

Although reconfiguration of Genomic Medicine Services in NHS England in 2019 was intended to standardise availability and application of genomic testing in different clinical scenarios across England,<sup>39</sup> it is clear that variability in both access and use of different technologies exists between genomic laboratory hubs in England, not to mention between different nations within the UK. Lack of routine access to loss of heterozygosity analysis was flagged as one particular issue hampering comprehensive investigation of probands presenting with dMMR, such that individuals with dMMR tumours demonstrating a single somatic variant and uninformative germline genetic testing are managed in the same way as those individuals where no causative variants are identified in constitutional or tumour-derived DNA. This is likely inappropriate as a significant proportion of tumours in which a single relevant variant will also demonstrate loss of heterozygosity at the locus of interest. Inequitable access to, and application of, long-range PCR testing of *PMS2* was also identified as an issue, although consensus was reached that this should be implemented as part of standard testing for those individuals with tumours demonstrating isolated *PMS2* loss.

In 2023, the National Lynch Syndrome Registry was successfully implemented in England, where entry to the register requires a molecular confirmation of Lynch syndrome.<sup>40</sup> We did not discuss how we can prospectively follow probands with u-dMMR, but it is clear that such data are required to inform guidelines in the future. Furthermore, data are required to determine proportion of dMMR in non-colorectal/non-endometrial cancers accounted for by various somatic aetiologies, not to mention clinical and analytical sensitivity and specificity of available assays to detect underlying mechanisms of disease.

Data so far indicate that the number of patients with dMMR colorectal cancer appropriately referred for germline genetic testing is suboptimal,<sup>40 41</sup> and this issue may be even more pronounced for patients with those cancers less commonly recognised as a Lynch syndrome-associated malignancy. Further training and education are required to ensure timely diagnosis of Lynch syndrome, and, where resources are limited, this may represent a more acute clinical risk than scenarios related to u-dMMR where significant genomic testing has already been undertaken.

### Further work

As assessment of MMR function continues to expand across different tumour types and constitutional testing of MMR genes increases in patients with non-colorectal/non-endometrial cancers, we hope to generate robust data regarding proportion of dMMR in such cancers accounted for by Lynch syndrome or alternative genomic mechanisms, by continuing and building on

previous collaborative work between NHS genomic laboratory hubs and National Disease Registration Service.<sup>42</sup> We appreciate, however, that a lack of uniformity in investigation of u-dMMR where clinical suspicion is low will hamper data collection for such cases—further emphasising the need for national collaboration to enable collection of at least some meaningful data. This information about the outcomes of testing, especially for non-colorectal/non-endometrial cancers, will help to inform diagnostic strategies, for example, clarifying the diagnostic yield of somatic and/or germline testing in these tumours.

Limitations of time at this meeting precluded discussion of gynaecological cancer risk management in probands with dMMR cancers and their at-risk relatives. Furthermore, we did not consider indications for consideration of constitutional mosaicism.

### CONCLUSION

While it is not possible to reach uniform advice for all u-dMMR cases, the authors hope that this article describes the difficulties and limitations encountered in clinical practice and provides the reader with support in clinical decision making for individual cases. Recommendations regarding extent of investigation and colorectal cancer surveillance for probands with dMMR cancers and their at-risk relatives should be made based on the best available evidence; but it is crucial that collection of evidence should be practicable in the face of limited resources (money, staff and time).

Despite efforts in standardising availability of comprehensive testing, inequity of access to the same is evident across the UK, hampering efforts to develop uniform guidance for best practice. As a compromise, if it is not reasonably feasible to perform comprehensive constitutional and tumour-based testing for every patient with dMMR cancer, bearing in mind local resources, identification of heritable risk factors in those patients where Mendelian cancer predisposition is most suspected should be prioritised. Consideration of further testing for cryptic constitutional MMR gene variants (currently only available on a research basis) should be considered where clinical suspicion of Lynch syndrome persists—for example, with those patients fulfilling Amsterdam II<sup>43</sup>/Revised Bethesda<sup>44</sup> criteria, or based on a high PREMM (PREdiction Model for gene Mutations)<sup>36</sup> score.<sup>20</sup> In such cases, Lynch-equivalent colonoscopic screening may be appropriate for FDRs of affected individuals.

Clinical judgement should be used to inform extent to which testing beyond standard testing is performed in families where clinical suspicion of such predisposition is lower. Importantly, clinical judgement should be used to inform surveillance recommendations in probands and their family members, and Lynch-equivalent surveillance is not always indicated where aetiology of dMMR remains elusive after reasonable efforts to determine the same.

We recommend that, at a minimum, ‘reasonable effort’ should include (where relevant), somatic *BRAF* testing and/or *MLH1* promoter hypermethylation testing and constitutional MMR gene testing (and additional genes if indicated as per national genomic testing directories), with further testing beyond that depending on clinical circumstances as outlined above.

Basic principles should also be considered, and it is important that performance and reporting of results from IHC and/or genomic testing be undertaken in line with best practice.<sup>45–47</sup>

### Author affiliations

<sup>1</sup>Cancer Genetics Unit, Royal Marsden Hospital NHS Trust, London, UK

<sup>2</sup>The Institute of Cancer Research, London, UK



<sup>3</sup>St Mark's Academic Institute Polyposis Registry, Harrow, UK

<sup>4</sup>Imperial College London, London, UK

<sup>5</sup>Department of Clinical Genetics, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

<sup>6</sup>Department of Medical Genetics, University of Cambridge, Cambridge, UK

<sup>7</sup>University of Leeds, Leeds, UK

<sup>8</sup>Department of Histopathology, Leeds Teaching Hospitals NHS Trust, Leeds, UK

<sup>9</sup>Familial Cancer Clinic, Department of Gynaecology, University College London Hospitals NHS Foundation Trust, London, UK

<sup>10</sup>Southeast Scotland Genetics Service, Western General Hospital, Edinburgh, UK

<sup>11</sup>Peninsula Regional Genetics Service, Royal Devon University Healthcare NHS Foundation Trust, Exeter, UK

<sup>12</sup>Faculty of Health and Life Sciences, University of Exeter Medical School, Exeter, UK

X Terri Patricia McVeigh @mcveighterri and Helen Hanson @Helen\_Hanson1

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#### ORCID iDs

Terri Patricia McVeigh <http://orcid.org/0000-0001-9201-9216>

Helen Hanson <http://orcid.org/0000-0002-3303-8713>

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