
DIAGNEXIA
Remote Expert Pathology
Connecting the World
to Expert Pathologists
Through digital pathology, Diagnexia connects clients to a global network of renowned pathologists, reducing turnaround time while maintaining the highest quality of care.
To find out more visit
diagnexia.com

Histopathology

Histopathology 2021 DOI: 10.1111/his.14390

Lynch syndrome screening in colorectal cancer: results of a prospective 2-year regional programme validating the NICE diagnostics guidance pathway throughout a 5.2-million population

Nicholas P West, Niall Gallop, Danny Kaye, Amy Glover, Caroline Young, Gordon G A Hutchins, Scarlet F Brockmoeller, Alice C Westwood, Hannah Rossington, Philip Quirke & the Yorkshire Cancer Research Bowel Cancer Improvement Programme Group

Pathology and Data Analytics, Leeds Institute of Medical Research at St James's, University of Leeds, Leeds, UK

Date of submission 2 February 2021
Accepted for publication 15 April 2021
Published online *Article Accepted* 19 April 2021

West N P, Gallop N, Kaye D, Glover A, Young C, Hutchins G G A, Brockmoeller S F, Westwood A C, Rossington H & Quirke P on behalf of the Yorkshire Cancer Research Bowel Cancer Improvement Programme Group
(2021) *Histopathology*. https://doi.org/10.1111/his.14390

Lynch syndrome screening in colorectal cancer: results of a prospective 2-year regional programme validating the NICE diagnostics guidance pathway throughout a 5.2-million population

Aims: Screening all patients newly diagnosed with colorectal cancer (CRC) for possible Lynch syndrome (LS) has been recommended in the United Kingdom since the National Institute for Health and Care Excellence (NICE) released new diagnostics guidance in February 2017. We sought to validate the NICE screening pathway through a prospective regional programme throughout a 5.2-million population during a 2-year period.

Methods and results: Pathology departments at 14 hospital trusts in the Yorkshire and Humber region of the United Kingdom were invited to refer material from patients with newly diagnosed CRC aged 50 years or over between 1 April 2017 and 31 March 2019 for LS screening. Testing consisted of immunohistochemistry for MLH1, PMS2, MSH2 and MSH6 followed by BRAF mutation analysis ± MLH1 promoter methylation testing in cases showing MLH1 loss. A total of 3141 individual specimens were submitted for testing from 12 departments consisting of 3061 unique tumours and 2791 prospectively acquired patients with CRC. Defective mismatch repair (dMMR) was observed in 15% of cases. In cases showing MLH1 loss, 76% contained a detectable BRAF mutation and, of the remainder, 77% showed MLH1 promoter hypermethylation. Of the patients included in the final analysis, 81 (2.9%) had an indication for germline testing.

Conclusion: LS screening using the NICE diagnostics guidance pathway is deliverable at scale identifying significant numbers of patients with dMMR. This information is used to refer patients to regional clinical genetics services in addition to informing treatment pathways including the use of adjuvant/neoadjuvant chemotherapy and immunotherapy.

Keywords: colorectal cancer, Lynch syndrome, mismatch repair

Address for correspondence: Nicholas P West, Pathology and Data Analytics, Level 4, Wellcome Trust Brenner Building, St James's University Hospital, Beckett Street, Leeds LS9 7TF, UK. e-mail: n.p.west@leeds.ac.uk

Introduction

Approximately 3% of colorectal cancers (CRC) arise in the context of Lynch syndrome (LS), where the patient has a germline mutation in a DNA mismatch repair (MMR) gene.[1] Prior to February 2017, CRC patients were tested for LS if they were at high risk according to clinical criteria, e.g. aged under 50 years or with a strong family history. However, a large proportion of LS patients were missed by this strategy. LS patients with CRC are at an increased risk of developing metachronous colorectal and other cancers. After an LS diagnosis, germline testing is offered to family members and screening/risk-reducing strategies used to identify early tumours or prevent their development, thereby improving outcomes.

The National Institute for Health and Care Excellence (NICE) issued new diagnostics guidance (DG27) in February 2017 recommending that all patients with newly diagnosed CRC be screened for LS.[2] The pathway includes testing tumour tissue for defective MMR (dMMR) by either microsatellite instability (MSI) testing or immunohistochemistry (IHC) for the MMR proteins MLH1, PMS2, MSH2 or MSH6. Tumours showing MSI or MLH1 loss should subsequently undergo BRAF mutation testing followed by MLH1 promoter methylation analysis in the absence of a BRAF mutation. Patients with tumours showing MSH2, MSH6 or isolated PMS2 loss, or MLH1 loss/MSI with no evidence of BRAF mutation/MLH1 promoter hypermethylation, are referred for germline testing if clinically appropriate.

The Yorkshire Cancer Research Bowel Cancer Improvement Programme (YCR BCIP) commenced in 2016 with an aim to improve CRC outcomes among the 16 CRC multidisciplinary teams within 14 NHS trusts in the Yorkshire and Humber (YH) region of the United Kingdom, serving a population of 5.2 million.[3] The programme included funding to implement LS screening until a point where the service could be commissioned through the NHS. In this study, we report the results of this prospective 2-year programme as a validation of the DG27 IHC pathway.

Materials and methods

CASES FOR TESTING

LS screening was offered to all CRC multidisciplinary teams in the YH region. All patients diagnosed with adenocarcinoma of the colon or rectum between 1 April 2017 and 31 March 2019 for whom there was no routine LS screening pathway already in place were eligible for testing. It was anticipated that pathways were in place to screen patients diagnosed under the age of 50 years following the Royal College of Pathologists 2014 guidance,[4] and the programme sought to not disrupt these established pathways. Participating centres sent a single formalin-fixed paraffin-embedded tissue block per unique tumour along with a completed request form and local histopathology report to the central laboratory at the University of Leeds. The diagnostic biopsy block (or polypectomy if a malignant polyp) was recommended for testing to reduce the risks of poor fixation-associated artefacts and effects of neoadjuvant treatment. However, the resection specimen was tested if this was the only specimen with invasive adenocarcinoma available.

TESTING PATHWAY

IHC was performed for the four MMR proteins (MLH1, PMS2, MSH2 and MSH6) on a Dako Autostainer (Dako, Glostrup, Denmark). Cases showing loss of MSH2, MSH6 or isolated PMS2 were recommended for germline testing if clinically appropriate. Cases showing loss of MLH1 underwent BRAF codon 600 mutational analysis by pyrosequencing.[5] Cases undergoing BRAF testing with no evidence of a mutation underwent MLH1 promotor methylation analysis in the Genetics Laboratory at Leeds Teaching Hospitals NHS Trust. Further details are provided in the Supporting information.

ETHICAL APPROVAL

Ethical approval was not required, because DG27 was already in place recommending LS screening in all patients diagnosed with CRC. Testing was considered part of the 'standard of care' clinical pathway. Specific patient consent was therefore not obtained.

STATISTICAL ANALYSIS

Categorical variables were compared using Pearson's c^2 test and continuous variables using the Mann–Whitney *U*-test. All analyses were performed using IBM SPSS Statistics version 23 (IBM, New York, NY, USA). Statistical significance was defined by any analyses where $P < 0.05$.

Results

CASES

Between 19 May 2017 and 14 May 2019, 3141 individual specimens were submitted for LS screening

from 12 histopathology departments throughout the YH region. Eighty specimens consisted of duplicate samples from the same tumour, e.g. biopsy followed by resection or two separate resection blocks. In most cases such duplicate samples were intentional, e.g. to complete the full testing pathway if the initial sample was exhausted or if the assay failed. Occasionally, duplicate samples were submitted in error. The combined results from any duplicate samples were used for the final analysis.

Of the 3061 unique tumours received for testing, 213 specimens were excluded from the final analysis (further details provided in the Supporting information). In total, 2791 patients with confirmed adenocarcinoma of the colon or rectum aged 50 years or over at diagnosis were screened. Fifty-three of these patients had synchronous tumours sent for testing (50 with two separate tumours and three with three separate tumours) and one had two separate metachronous tumours, resulting in 2848 individual CRCs tested and included in the final analysis.

A total of 1753 tests were performed on primary tumour biopsies (61.6%), 923 on primary tumour resections (32.4%), 153 on polypectomy specimens (5.4%), five on lymph node metastases (0.2%) and 14 on distant metastases (0.5%).

MISMATCH REPAIR STATUS

Immunohistochemistry (IHC) failed to give a definitive result in seven tumours (0.25%). For the 2841 tumours with a definitive IHC result, 2411 showed proficient MMR (pMMR) and 430 showed dMMR (15.1%), including 363 with primary MLH1 loss (12.8%), 25 with primary PMS2 loss (0.9%), 27 with primary MSH2 loss (1.0%) and 15 with primary MSH6 loss (0.5%).

Of the 363 tumours showing loss of MLH1/PMS2, BRAF and/or MLH1 promoter methylation testing failed to determine whether the features were probably in keeping with somatic dMMR in 12 cases (six due to methylation failure, two due to BRAF failure and four where no tumour remained after initial IHC). In these 12 cases, no alternative samples were available to complete the testing pathway. Of the other 351 cases showing MLH1/PMS2 loss, 267 (76.1%) demonstrated a BRAF V600E mutation. Of the 84 tumours with no evidence of BRAF mutation, 65 (77.4%) showed hypermethylation of the MLH1 promotor region.

Of 430 dMMR tumours, 44 (10.2%) showed unusual patterns of protein loss not described in DG27. These included loss of additional non-paired proteins, clonal loss and patchy loss (Figure 1). Further details regarding unusual patterns of protein loss and synchronous/metachronous tumours are provided in the Supporting information.

RELATIONSHIP BETWEEN MMR STATUS AND CLINICOPATHOLOGICAL VARIABLES

The relationship between MMR status and clinicopathological variables is shown in Table 1. When the dMMR tumours were assessed by the primary protein lost by IHC, the association with female gender and older age was only observed in the MLH1 loss cases (Table 2). A detailed analysis by tumour site showed that there was a significant difference in the distribution of pMMR tumours, probably somatic dMMR and possible germline dMMR (all $P < 0.0001$, Table 3 and Figure 2). While cases showing MLH1 loss (both somatic and possible germline) and isolated PMS2 loss were predominantly right-sided, there was a more even distribution for cases showing MSH2 loss and a left-sided predominance for isolated MSH6 loss (Table 2).

RECOMMENDATION FOR GERMLINE TESTING

Of the cases in the final analysis, 85 (2.98%) tumours from 81 (2.90%) patients had a definite indication for germline testing according to DG27. This did not include cases showing MSH2 and/or MSH6 loss in the context of MLH1/PMS2 loss with either a BRAF mutation or MLH1 promoter hypermethylation. A further six patients were recommended for germline testing due to failure of MLH1 methylation analysis in the context of MLH1/PMS2 loss with wild-type BRAF and no remaining material available to test.

Discussion

This is the largest regional validation study of the NICE DG27 IHC pathway for LS screening, to our knowledge, resulting in more than 3000 individual tumours tested from a 5.2-million population during a 2-year period. The programme prospectively tested tumour material from 2791 patients with CRC diagnosed at the age of 50 years or more using MMR IHC as a first-line test followed by BRAF mutational analysis and MLH1 promoter methylation, as required.

Two-thirds of cases were tested using diagnostic biopsy or polypectomy specimens, which represented the first available sample, and ensured that the MMR

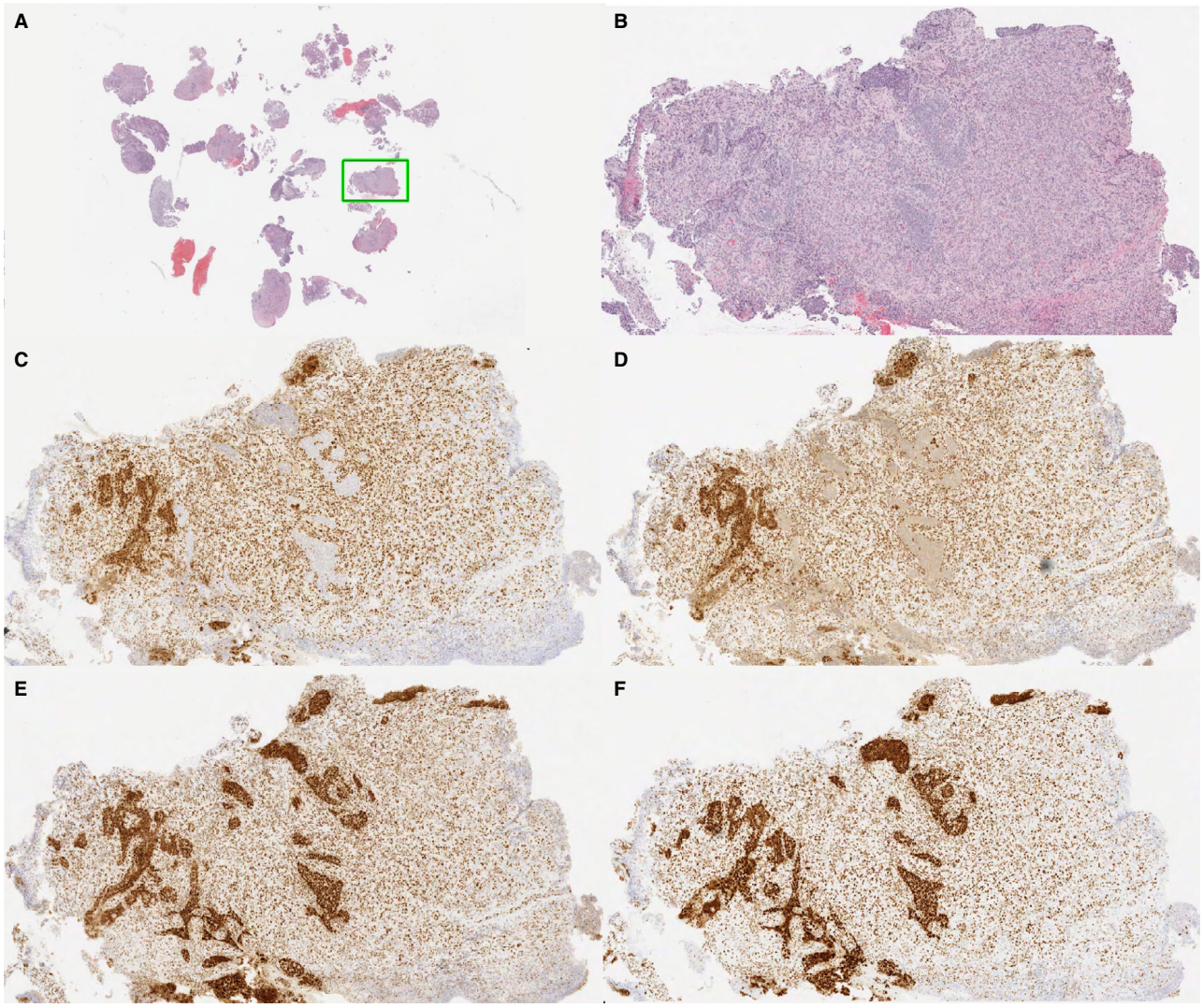

Figure 1. An unusual case at the rectosigmoid junction showing clonal defective mismatch repair (dMMR) (MLH1 and PMS2) in the invasive carcinoma. This was associated with no evidence of BRAF mutation; however, hypermethylation of the MLH1 promoter region was demonstrated, in keeping with somatic dMMR. **A,** Overview haematoxylin and eosin (H&E) stain of the multiple biopsy pieces with rectangular annotation indicating the piece shown at higher magnification. **B,** Higher magnification H&E stain showing invasive carcinoma. **C,** Corresponding area stained with MLH1 showing tumour with areas of retained expression (left) and lost expression (right). **D,** Corresponding area stained with PMS2 showing tumour with areas of retained expression (left) and lost expression (right). **E,** Corresponding area stained with MSH2 showing tumour with retained expression throughout. **F,** Corresponding area stained with MSH6 showing tumour with retained expression throughout.

result was reported to the multidisciplinary team at an early stage in the treatment pathway. This is increasingly important for management outside LS screening, given the better prognosis of dMMR tumours, relative insensitivity to 5-fluorouracil based regimens and sensitivity to immunotherapy.[7–9] In the YH region, MMR status forms a key component of the adjuvant chemotherapy decision-making algorithm,[10] and immunotherapy is being considered in stage IV dMMR, in line with the NHS England interim treatment options during the COVID-19 pandemic.[11] We have introduced a neoadjuvant chemotherapy pathway for advanced operable colon cancer following the FOxTROT trial, and again MMR status forms an important component, given the limited tumour regression and no apparent survival benefit in dMMR tumours.[12] Other key advantages of testing biopsies include avoiding artefacts related to poor fixation and the neoadjuvant therapy, which can affect MSH6 staining in a proportion of cases.[13]

Table 1. Relationship between clinicopathological variables and MMR status

	pMMR (n = 2411)	dMMR (n = 430)	P-value
Gender			
Male	1493 (62.9)	151 (36.1)	< 0.0001
Female	879 (37.1)	267 (63.9)	
Not stated	39	12	
Age (years)			
Median	71	76	< 0.0001
IQR	63 to 78	68 to 82	
Site			
Right side	670 (28.1)	343 (80.7)	< 0.0001
Left side	1713 (71.9)	82 (19.3)	
Not stated	28	5	
Tumour type			
Adenocarcinoma NOS	2312 (96.1)	388 (90.7)	< 0.0001
Mucinous adenocarcinoma	86 (3.6)	36 (8.4)	
Signet ring cell carcinoma	7 (0.3)	3 (0.7)	
Medullary carcinoma	0 (0.0)	1 (0.2)	
Not stated	6	2	
Differentiation			
Well/moderate	2176 (94.1)	286 (72.6)	< 0.0001
Poor	137 (5.9)	108 (27.4)	
Not stated	98	36	
pT stage			
pT1	56 (7.3)	6 (1.4)	0.389
pT2	129 (16.9)	29 (19.9)	
pT3	338 (44.2)	69 (47.3)	
pT4	240 (31.4)	42 (28.8)	
Not stated	1648	284	
Tumour size (mm)			
Median	38	55	< 0.0001
IQR	28 to 50	36 to 70	
pN stage			
pN0	408 (54.8)	99 (68.8)	0.007
pN1	212 (28.5)	30 (20.8)	
pN2	125 (16.8)	15 (10.4)	
Not stated	1666	286	
Lymph node yield			
Median	18	21	< 0.0001
IQR	13 to 24	15 to 29	

Values in parentheses represent percentages. pMMR, proficient mismatch repair; dMMR, defective mismatch repair; IQR, interquartile range; NOS, not otherwise specified. Data regarding stage, tumour size and lymph node yield are restricted to cases where resection specimens were tested.

In the remaining third of cases testing was largely performed on primary tumour resections, because no biopsy was taken, biopsy was non-diagnostic, adenocarcinoma cut out of the biopsy block or biopsy testing was missed.

IHC was chosen over MSI testing due to the lower failure rate, ability to test samples with tiny amounts of tumour (common in biopsies), determine the specific proteins affected and whether clonal/complete and rapid turnaround of the result. By contrast, MSI testing generally requires a tumour content of > 20% (depending on technology), cannot determine the affected genes (or whether clonal) and requires sending away to a centralised genomics laboratory leading to significantly increased turn-around times. Discussion with our regional clinical genetics service confirms significant value in knowing the probable gene affected when interrogating the germline for potential novel mutations. In addition, IHC is readily available within most histopathology departments and could be easily rolled out into clinical practice internationally if resources are identified.

In patients where MMR testing was successful the dMMR rate was 15.1%, with a recommendation for germline testing in 2.9% of patients overall. These data are in keeping with previous studies.[14] As expected, the majority of dMMR cases showed MLH1/PMS2 loss with either a BRAF mutation or MLH1 promoter hypermethylation, in keeping with somatic dMMR. Despite this, a number of cases

Table 2. Relationship between clinicopathological variables and MMR status

	MLH1 loss (n = 363)	PMS2 loss (n = 25)	MSH2 loss (n = 27)	MSH6 loss (n = 15)	P-value
Gender					
Male	107 (30.2)	15 (62.5)	18 (72.0)	11 (73.3)	< 0.0001
Female	247 (69.8)	9 (37.5)	7 (28.0)	4 (26.7)	
Not stated	9	1	2	0	
Age (years)					
Median	77	64	62	68	< 0.0001
IQR	70 to 82	60 to 72	56 to 67	61 to 75	
Site					
Right side	305 (85.2)	19 (76.0)	15 (55.6)	4 (26.7)	< 0.0001
Left side	53 (14.8)	6 (24.0)	12 (44.4)	11(73.3)	
Not stated	5	0	0	0	
Tumour type					
Adenocarcinoma NOS	330 (91.4)	23 (92.0)	20 (74.1)	15 (100.0)	0.153
Mucinous adenocarcinoma	27 (7.5)	2 (8.0)	7 (25.9)	0 (0.0)	
Signet ring cell carcinoma	3 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	
Medullary carcinoma	1 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	
Not stated	2	0	0	0	
Differentiation					
Well/moderate	239 (71.6)	18 (78.3)	17 (77.3)	12 (80.0)	0.751
Poor	95 (28.4)	5 (21.7)	5 (22.7)	3 (20.0)	
Not stated	29	2	5	0	
pT stage					
pT1	6 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.924
pT2	24 (19.8)	1 (10.0)	3 (25.0)	1 (33.3)	
pT3	56 (46.3)	6 (60.0)	5 (41.7)	2 (66.7)	
pT4	35 (28.9)	3 (30.0)	4 (33.3)	0 (0.0)	
Not stated	242	15	15	12	
Tumour size (mm)					
Median	55	55	64	30	0.319
IQR	38 to 70	35 to 73	30 to 81	30 to 30	

Table 2. (*Continued*)

	MLH1 loss (*n* = 363)	PMS2 loss (*n* = 25)	MSH2 loss (*n* = 27)	MSH6 loss (*n* = 15)	*P*-value
pN stage					
pN0	80 (67.2)	6 (60.0)	10 (83.3)	3(100.0)	0.697
pN1	25 (21.0)	3 (30.0)	2 (16.7)	0 (0.0)	
pN2	14 (11.8)	1 (10.0)	0 (0.0)	0 (0.0)	
Not stated	244	15	15	12	
Lymph node yield					
Median	22	22	17	16	0.411
IQR	15 to 30	17 to 29	13 to 24	14 to 16	

Values in parentheses represent percentages. IQR, interquartile range; NOS, not otherwise specified. Data regarding stage, tumour size and lymph node yield are restricted to cases where resection specimens were tested.

Table 3. Relationship between mismatch repair status and tumour location

	pMMR (*n* = 2411)	Probable somatic dMMR (*n* = 332)	Possible germline dMMR (*n* = 85)
Caecum	271 (11.4)	94 (28.7)	12 (14.1)
Ascending colon	202 (8.5)	104 (31.7)	21 (24.7)
Hepatic flexure	72 (3.0)	30 (9.1)	6 (7.1)
Transverse colon	123 (5.2)	56 (17.1)	10 (11.8)
Splenic flexure	44 (1.8)	9 (2.7)	8 (9.4)
Descending colon	90 (3.8)	14 (4.3)	9 (10.6)
Sigmoid/ rectosigmoid colon	723 (30.4)	15 (4.6)	7 (8.2)
Rectum	855 (35.9)	6 (1.8)	12 (14.1)
Missing	31	4	0

Values in parentheses represent percentages. pMMR, proficient mismatch repair; dMMR, defective mismatch repair. Probable somatic dMMR was defined as tumours showing MLH1/PMS2 loss with either a BRAF mutation or MLH1 promoter hypermethylation. Possible germline dMMR was defined as all cases in which a recommendation for germline testing was made in the final report.

showed more unusual IHC staining features that are not clearly defined in the DG27 flowchart, including cases with clonal loss and co-existing loss of non-paired proteins. It is important that histopathologists are aware of these patterns and report them appropriately to avoid unnecessary referrals for germline testing, with associated costs and distress for the patient and their family. We have previously described our approach to cases demonstrating the 'null phenotype',[15] and a similar approach is required for all cases showing unusual features based on the possibility and likelihood of underlying germline versus somatic origin. Cases showing MLH1/PMS2 loss with additional clonal MSH6 ± MSH2 loss in the context of a BRAF mutation or MLH1 promoter hypermethylation can be assumed to be entirely somatic in origin with no further testing required. Cases with an indication for germline testing in which there is evidence of co-existing loss of non-paired proteins do not need further testing; these should be recommended for clinical genetics referral. More attention is required in cases showing complete loss of MLH1/PMS2/MSH6 ± MSH2 in the context of a BRAF mutation or MLH1 promoter hypermethylation. If testing was initially performed on a biopsy specimen, it is recommended to repeat the IHC on multiple blocks from the resection to determine whether the additional loss of MSH6 ± MSH2 is complete or clonal. If confirmed to be complete, a clinical genetics referral is recommended due to the small risk of somatic MLH1 loss occurring on a background of a germline mutation in MSH2 or MSH6.[16]

Some authors have previously proposed a reduced cost approach whereby staining for only two IHC markers (PMS2 and MSH6) could be performed as an initial screen.[17] However, the presence of patchy MSH6 loss in 15% of our cases showing complete MSH2 loss suggests that this approach should be used

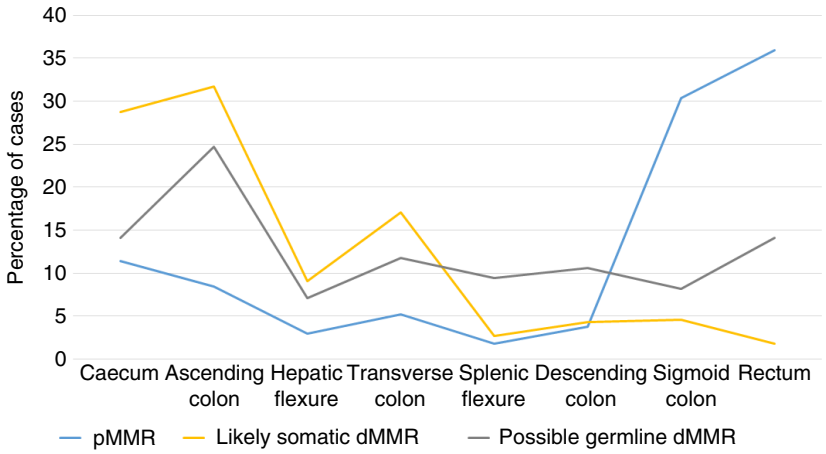

Figure 2. The distribution of cases within the large bowel showing proficient mismatch repair (pMMR) and defective mismatch repair (dMMR) according to whether the cause was likely to be somatic or possible germline in origin. Probable somatic dMMR was defined as tumours showing MLH1/PMS2 loss with either a BRAF mutation or MLH1 promoter hypermethylation. Possible germline dMMR was defined as all cases in which a recommendation for germline testing was made in the final report.

cautiously, especially where patchy staining might be interpreted as an artefact. Similarly, in patients with multiple tumours, testing only one tumour may miss dMMR in 11% of cases, although none of these cases showed features suggestive of germline origin.

The relationship between dMMR and key clinicopathological variables has previously been described, and we confirmed these known associations in our population.[18] There were key differences between dMMR cases showing probably somatic and possible germline origin, with those recommended for germline testing being younger, more likely to be male and evenly distributed around the large bowel. However, there were significant differences between the patterns of protein loss with regard to tumour site, specifically with MSH6 deficient cancers being more likely to be left-sided.

One of our key objectives was to engage with local commissioners to migrate testing to the routine clinical service. Despite DG27 being in place, it was shown in 2018 that only 17% of hospitals were screening all cases largely due to inadequate resources in histopathology.[19] While screening all patients at diagnosis is cost-effective, the savings may take several years to materialise and money often needs to move between budgets to ensure that histopathology departments are adequately resourced. While DG27 recognises the clinical and cost-effectiveness of screening all CRC patients for LS, it does not highlight the additional clinical and financial benefits of identifying somatic dMMR. Good engagement with our local Cancer Alliances during the programme enabled testing to migrate to the routine service from 1 April 2019.

With funding and capacity being a key limitation internationally, the possibility of low-cost high-throughput technologies may be of benefit. Artificial intelligence applied to digitally scanned H&E slides has been shown to accurately predict dMMR with 95% sensitivity.[20,21] Digital pathology is increasingly used in routine practice, thus the costs are anticipated to be minimal, if validated for clinical practice. Others have proposed a low-cost next-generation sequencing approach to MSI detection with the inclusion of BRAF testing to streamline the pathway.[22]

There are some recognised limitations within our programme. Hospitals joined the programme at various times, with some taking several months to submit cases. A small number did not submit any cases for testing. Other centres commenced testing in-house or at an alternative external centre before the programme completed. Thus, a number of CRCs diagnosed across the region were not tested through the programme. Based on the expected number of cancers, it is estimated that 42% of all newly diagnosed CRC cases were tested through the programme. Secondly, we did not have consent to collect data regarding the number of patients referred for germline testing and the results. Thus, we do not know the number of new LS patients detected directly through the programme or indirectly through testing family members.

In conclusion, we have shown through a prospective regional programme throughout a 5.2-million

population during a 2-year period that the NICE diagnostics guidance DG27 for LS screening in CRC is deliverable at scale, with more than 3000 tumours centrally tested, identifying 15% of patients with dMMR and 2.9% requiring germline testing. In addition to screening for LS, the results are being used to inform treatment pathways, including the use of chemotherapy in the adjuvant/neoadjuvant setting and the use of immunotherapy in stage IV. We have shown that such a programme can be undertaken in routine clinical practice following engagement with local commissioners through the Cancer Alliances to ensure that histopathology departments are adequately funded and resourced.

Acknowledgements

The YCR BCIP management group includes Professor Phil Quirke, Prof Eva Morris, Hannah Rossington, Dr Nick West, Professor Paul Finan, Dr Damian Tolan, Dr Dan Swinson, Professor David Sebag-Montefiore, Dr Penny Wright, Professor Matt Seymour, Dr Amy Glover, Aidan Hindley, John Taylor and Jackie Mara. The histological and immunohistochemical processing of samples was performed by Subaashini Natarajan, Danny Kaye, Jonathan Davis, Niall Gallop and Phillipa Vaughn-Beaucaire. Pyrosequencing was performed by Gemma Hemmings and interpreted by Susan Richman and Henry Wood. The MLH1 promoter methylation testing was contracted to the Leeds Teaching Hospitals NHS Trust Genetics Laboratory. We thank the histopathologists from the following hospitals who have engaged with the YCR BCIP Lynch syndrome screening programme: Airedale NHS Foundation Trust, Barnsley Hospital NHS Foundation Trust, Bradford Teaching Hospitals NHS Foundation Trust, Calderdale and Huddersfield NHS Foundation Trust, Doncaster and Bassetlaw Teaching Hospitals NHS Foundation Trust, Harrogate and District NHS Foundation Trust, Leeds Teaching Hospitals NHS Trust, Mid Yorkshire Hospitals NHS Trust, The Rotherham NHS Foundation Trust, Northern Lincolnshire and Goole NHS Foundation Trust and Sheffield Teaching Hospitals NHS Foundation Trust. The Yorkshire Cancer Research Bowel Cancer Improvement Programme (YCR BCIP) is funded by Yorkshire Cancer Research, Harrogate, UK (grant number L394). P.Q. is a National Institute for Health Research Senior Investigator. C.Y. and S.F.B. hold National Institute for Health Research Clinical Lectureships. A.C.W. is funded through a CRUK and Stella Erdheim PhD fellowship.

Conflict of interests

The authors have no conflicts of interest to declare.

References

1. Moreira L, Balaguer F, Lindor N *et al*. Identification of Lynch syndrome among patients with colorectal cancer. *JAMA* 2012; **308**; 1555–1565.
2. National Institute for Health and Care Excellence. Molecular testing strategies for Lynch syndrome in people with colorectal cancer. Diagnostics guidance [DG27]. Available at: https://www.nice.org.uk/guidance/dg27 (accessed 29 December 2020).
3. Taylor J, Wright P, Rossington H *et al*. Regional multidisciplinary team intervention programme to improve colorectal cancer outcomes: study protocol for the Yorkshire Cancer Research Bowel Cancer Improvement Programme (YCR BCIP). *BMJ Open* 2019; **9**; e030618.
4. Loughrey MB, Quirke P, Shepherd NA. *Dataset for colorectal cancer histopathology reports*. London: Royal College of Pathologists, 2014.
5. Richman SD, Adams R, Quirke P *et al*. Pre-trial inter-laboratory analytical validation of the FOCUS4 personalised therapy trial. *J. Clin. Pathol.* 2016; **69**; 35–41.
6. Helliwell T, Dojcinov S, Cree I, Rous B, Eden M, Cross S. *Standards for integrated reporting in cellular pathology*. London: Royal College of Pathologists, 2017.
7. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J. Clin. Oncol.* 2005; **23**; 609–618.
8. Sargent DJ, Marsoni S, Monges G *et al*. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J. Clin. Oncol.* 2010; **28**; 3219–3226.
9. Chalabi M, Fanchi LF, Dijkstra KK *et al*. Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. *Nat. Med.* 2020; **26**; 566–576.
10. Taylor JC, Swinson D, Seligmann JF *et al*. Addressing the variation in adjuvant chemotherapy treatment for colorectal cancer: can a regional intervention promote national change? *Int. J. Cancer* 2021; **148**; 845–856.
11. National Institute for Health and Care Excellence. COVID-19 rapid guideline: delivery of systemic anticancer treatments. NICE guideline [NG161]. Available at: https://www.nice.org.uk/guidance/ng161 (accessed 29 December 2020).
12. Seligmann JF; on behalf of the FOxTROT Collaborative Group. FOxTROT: neoadjuvant FOLFOX chemotherapy with or without panitumumab (Pan) for patients (pts) with locally advanced colon cancer (CC). *J. Clin. Oncol.* 2020; **38**(Suppl. 15); 4013.
13. Kuan SF, Ren B, Brand R, Dudley B, Pai RK. Neoadjuvant therapy in microsatellite-stable colorectal carcinoma induces concomitant loss of MSH6 and Ki-67 expression. *Hum. Pathol.* 2017; **63**; 33–39.
14. Cerretelli G, Ager A, Arends MJ, Frayling IM. Molecular pathology of Lynch syndrome. *J. Pathol.* 2020; **250**; 518–531.
15. Westwood A, Glover A, Hutchins G *et al*. Additional loss of MSH2 and MSH6 expression in sporadic deficient mismatch repair colorectal cancer due to MLH1 promoter hypermethylation. *J. Clin. Pathol.* 2019; **72**; 443–447.
16. Hagen CE, Lefferts J, Hornick JL, Srivastava A. 'Null pattern' of immunoreactivity in a Lynch syndrome-associated colon

cancer due to germline MSH2 mutation and somatic MLH1 hypermethylation. *Am. J. Surg. Pathol.* 2011; **35**; 1902–1905.
17. Hicks SC, Ward RL, Hawkins N. Immunohistochemistry for PMS2 and MSH6 alone can replace a four-antibody panel for mismatch repair deficiency screening in colorectal adenocarcinoma. *Pathology* 2011; **43**; 84–85.
18. Hutchins G, Southward K, Handley K *et al*. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J. Clin. Oncol.* 2011; **29**; 1261–1270.
19. Bowel Cancer UK. Time to test. (accessed 29 December 2020) Available at: https://bowelcancerorguk.s3.amazonaws.com/Campaigns/LYNCH%20SYNDROME%20REPORT%20FINAL.pdf (accessed 29 December 2020).
20. Kather JN, Pearson AT, Halama N *et al*. Deep learning can predict microsatellite instability directly from histology in gastrointestinal cancer. *Nat. Med.* 2019; **25**; 1054–1056.
21. Echle A, Grabsch HI, Quirke P *et al*. Clinical-grade detection of microsatellite instability in colorectal tumors by deep learning. *Gastroenterology* 2020; **159**; 1406–1416.
22. Gallon R, Sheth H, Hayes C *et al*. Sequencing-based microsatellite instability testing using as few as six markers for high-throughput clinical diagnostics. *Hum. Mutat.* 2020; **41**; 332–341.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Method S1. Detailed information regarding the testing pathways and methodology.

Result S1. Results from patients with synchronous/metachronous tumours and unusual staining patterns.