Diagnosis of Lynch Syndrome

Subjects: Pathology

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International guidelines for the diagnosis of Lynch syndrome (LS) recommend molecular screening of colorectal cancers (CRCs) to identify patients for germline mismatch repair (MMR) gene testing.

Lynch syndrome screening mismatch repair deficiency

1. Introduction

Lynch syndrome (LS) is the genetic predisposition to cancer in a variety of organs, in particular those of the gastrointestinal and genitourinary systems, caused by a germline pathogenic variant affecting one of four mismatch repair (MMR) genes: *MLH1*, *MSH2*, *MSH6*, or *PMS2* [1]. The most prevalent cancers within the Lynch spectrum are colorectal cancer (CRC) and endometrial cancer (EC), with cumulative incidences up to 57.1% and 48.9% by age 75 years, respectively, based on data from the Prospective Lynch Syndrome Database (PLSD) [2]. Known LS gene carriers can benefit from personalised cancer treatment, cancer surveillance, and cancer prophylaxis, including colorectal and gynaecological surgery and daily aspirin intake [1][3][4][5][6]. Hence, their identification is critical to optimise their clinical management. Since the discovery of its genetic cause in the 1990s, the strategies to identify LS have evolved with the advance of our knowledge of its phenotype and the diagnostic technologies available. However, LS is vastly underdiagnosed, with estimates that it is as common as one in 279 of the general population (*MLH1* = one in 1946, *MSH2* = one in 2841, *MSH6* = one in 758, and *PMS2* = one in 714) [7], and accounts for approximately 3% of CRCs and ECs [8][9]. Nearly three decades since its genetic definition, which have seen the completion of the Human Genome Project and the development of 2nd- and 3rd-generation sequencing technologies, a reappraisal of current LS screening strategies, and consideration of how these may evolve, is timely. Furthermore, the emergence of immunotherapy based on PD-L1 blockade has brought new impetus to the field, with pembrolizumab being the first site-agnostic agent to be licenced for cancer treatment. This has made identification of MMR-deficient tumours, whether sporadic or hereditary [3], a growing need in patient care.

2. Defining LS: A History

The first clinical description of LS was Aldred Scott Warthin's "Family G" in 1913. Thirty three of its 70 members had been diagnosed with colonic, endometrial, or gastric cancer, suggesting an autosomal dominant inheritance of increased cancer risk [10]. Through the 1960s, 1970s and 1980s, Henry T. Lynch published a series of cancer families, including a follow-up description of Family G [11], and gave LS its first widely accepted name: Hereditary Non-Polyposis Colorectal Cancer (HNPCC). HNPCC was defined to identify families for linkage analysis, and to highlight the lack of polyposis in the colorectum in contrast with the well-described CRC syndrome Familial

Adenomatous Polyposis (FAP). It was further subdivided into Lynch syndromes I and II depending on the tumour spectrum in the family [12].

In 1993, the underlying genetic cause of LS was discovered. Analysis of polymorphic microsatellites (short tandem repeat sequences) to detect loss of heterozygosity revealed that approximately 15% of CRCs have an exceptionally high frequency of microsatellite insertion and deletion variants (indels), a phenotype designated high microsatellite instability (MSI-H) or replication error positive. These MSI-H CRCs were diploid (unlike the majority of CRCs that show chromosomal instability), were associated with HNPCC, had a better prognosis, and tended to be proximally located (right sided) with poor cellular differentiation and increased immune cell infiltration [13][14][15][16]. Concurrently, in vivo experiments in yeast showed that loss-of-function mutations in MMR genes *MLH1*, *MSH2* and *PMS1* caused an MSI-H phenotype [17]. The causative link between MMR gene defects and LS was then established using the yeast *MSH2* gene to map human *MSH2* to chr2 p16-p21 and identify a pathogenic variant segregating with MSI-H HNPCC [18][19]. Pathogenic variants were subsequently found in the other MMR genes throughout the 1990s, including *MLH1* [20], *PMS2* [21], and *MSH6* [22]. In 2009, it was shown that 3′ deletions in *EPCAM* also cause LS through methylation of the deletion locus and silencing of the neighbouring *MSH2* gene [23]. Sporadic MSI-H CRCs were shown to be associated with promoter methylation (and therefore silencing) of *MLH1* [24][25]. The term HNPCC has been replaced by “Lynch” syndrome to recognise the risk for a broad spectrum of tumours beyond CRC, and to unify Lynch syndromes I and II by their shared genetic aetiology [26].

3. Current Clinical Guidance for LS Screening

In 2017, the UK National Institute of Health and Care Excellence (NICE) published their Diagnostic Guidance 27, stating that all CRC patients, irrespective of age or other clinical features, should be screened for LS [27]. The multistep screening pipeline begins with molecular analysis of the CRC. MMR deficiency testing of the tumour, by MSI analysis or immunohistochemistry (IHC) to show loss of MMR protein expression, is used to identify potential LS-associated CRCs. The utility of MMR deficiency testing is based on the repeated observation that nearly all LS CRCs are MMR deficient [28][29][30][31], following somatic loss of function of the second allele of the germline-affected MMR gene according to Knudson’s two-hit hypothesis [32]. Subsequently, MMR-deficient CRCs are tested for *BRAF* c.1799T>A (p.V600E) variants and *MLH1* promoter methylation to improve screening specificity, as both are associated with sporadic tumours ($p < 0.001$) [33][34][35][36]. Patients with MMR-deficient CRCs lacking *BRAF* c.1799T>A (p.V600E) variants and *MLH1* promoter methylation are selected for germline MMR gene testing [27]. Similar screening guidelines have been published by the American Society for Clinical Pathology [37], and the European Society for Medical Oncology [38].

These guidelines are based on decades of evidence that show molecular tumour analysis is a superior screening strategy to select CRC patients for germline MMR gene testing compared to screening by familial or clinical criteria [1]. For example, pooled data from four large cohorts of unselected CRC patients ($n = 3671$), consulted between 1994 and 2010, found that the Bethesda Guidelines, which screen by familial and clinical criteria followed by molecular tumour analysis [39][40], had 87.8% sensitivity and 97.5% specificity for LS gene carrier detection, whereas screening by universal molecular tumour analysis had 100% sensitivity and 93.0% specificity [8]. Cost-

effectiveness analyses, which balance the cost of patient screening and cascade testing of family members against the benefits of cancer surveillance and prophylaxis, further support screening strategies based on molecular analysis of CRCs [41][42][43]. A study comparison has shown general agreement of this cost-effectiveness between different countries [44]. An assumption made by these cost-effectiveness analyses is that the identification of LS probands and asymptomatic relatives who carry the LS variant will reduce cancer burden and costs due to prevention, surveillance and early detection [43]. Therefore, it is of critical importance to the clinical utility of LS screening that healthcare infrastructure has the capacity to offer these interventions to all identified LS gene carriers.

The implementation of LS screening in clinical practice has had mixed efficacy. A systematic review in 2017 identified five studies assessing the clinical performance of LS screening of CRC patients. Three of the five studies used a universal molecular screening approach, and two preselected patients based on clinical and familial criteria prior to molecular analyses of the tumour. The frequency of LS diagnoses ranged from 0.0% (0/31) to 5.3% (3/57) [45]. The largest study employing universal screening had an LS detection rate of 2.2% (17/784) [46], which suggests that approximately 73% of LS gene carriers were identified assuming 3% of the cohort were carriers. This shows current diagnostic guidance can be effective in a clinical setting. However, in the US, between 2010 and 2012, only 28.2% (43,143/152,993) and 43.1% (7422/17,218) of CRC patients aged below 70 and 50 years, respectively, were tested for tumour MMR deficiency [47], despite concurrent estimates that only 1.2% of LS gene carriers were known to clinical services [48]. Similarly, estimates from the UK suggest that, in 2016, routine screening for LS in CRC patients aged below 50 years was not performed in 29.5% (46/156) of UK hospitals [49] despite guidance promoting this from the UK Royal College of Pathologists [50]. Given these observations, here, we review the key barriers to implementation, as well as the limitations, of current LS screening guidelines. Following this we discuss the advances in our technology and knowledge that may further improve LS identification by addressing these barriers and limitations, or providing new screening opportunities.

4. Barriers to Implementing LS Screening Guidance

A US survey of 509 clinicians belonging to the American College of Gastroenterology found that the most common reasons given for a lack of MMR deficiency testing of CRCs to screen for LS were: prohibitive cost (33.3%), unfamiliarity interpreting results (29.2%), unavailable genetic counselling (24.9%), and unavailable germline genetic testing (20.0%) [51]. Similarly, cost, practical limitations, resources, and genetic counsellor availability were the key limitations identified in UK hospitals [49] and among Canadian genetic counsellors and pathologists [52]. These surveys show that the follow up of patients with a CRC suggestive of LS has barriers that are equally important to address as the barriers to universal molecular analysis of CRCs. This is also evident in clinical studies assessing the efficacy of LS screening in practice. For example, in a study of 1612 CRCs tested for MMR deficiency during the period 2004–2013, only 29.9% (82/274) of patients with a MMR-deficient tumour were subsequently consulted at a familial cancer clinic, leading to a low yield of LS diagnoses at 0.6% (10/1612) [53]. Uptake of LS screening within different demographics, particularly the clinically underserved, may face additional barriers, including access to clinics, cultural beliefs around healthcare, and language barriers [54]. Indeed, an

analysis of CRC patient outcomes from 2012 to 2016 in four US centres showed that, whilst there was no difference in tumour MMR deficiency testing uptake or results, African American and Hispanic patients were significantly less likely to be referred for genetic counselling and testing [55]. Specialist hospitals setup to address such specific needs have been shown to provide high-quality LS screening to >90% of patients irrespective of their background [54]. However, a concerted effort to overcome both general and demographic-specific barriers will be needed for fair and widespread LS screening to be achieved. The suggestion of dedicated LS screening programs has had strong support from healthcare professionals [56]. Careful program and policy design, an interdisciplinary approach, and sustained funding have been highlighted as the key requirements of a successful LS screening program [52].

Stakeholder education will be an important consideration when deploying LS screening programs. A 2016 survey of UK National Health Service General Practitioners found that 29.2% (294/1007) were not aware of LS (including by its alternative names such as HNPCC), and only 46.7% of those who had heard of LS were aware of the reduction in CRC risk associated with daily aspirin intake [57]. Another survey found that 41% (82/201) of US medical students did not know of LS. Of the students who had heard of LS, only 46% knew its genetic aetiology, only 23% knew of screening criteria to identify LS, and only 32% and 17% knew of recommendations for CRC and EC surveillance, respectively [58]. A lack of knowledge of the LS phenotype and clinical management was also observed in a 2014 survey of Australian healthcare providers from a variety of disciplines likely to encounter an LS patient: 7.7% (11/144) were unfamiliar with hereditary cancer syndromes in general, and 13.4% thought guidelines for LS screening were unavailable [59]. In this same study, the most frequently (55.6%; 79/142) identified barrier to referring suspected LS gene carriers for further testing was a lack of interest from the patient [59]. This suggests that even with an optimised and informed healthcare service, the uptake of LS screening may still be limited. Therefore, patients are also a key target of stakeholder education. In a randomised controlled trial of universal MMR deficiency testing of CRCs to screen for LS, compared to physician- or self-referral, a survey of the 145 participants' perspectives showed that more than 90% agreed that universal LS screening should be offered to CRC patients, and that they understood the reason for screening. Furthermore, when given a list of potential benefits of LS screening (for example, better understanding of hereditary CRC risk), 50.3% endorsed all eight benefits and 84.8% endorsed at least six benefits. Very low levels of anxiety due to screening were observed [60]. This is in contrast to the low patient interest observed by some healthcare practitioners [59]. The discrepancies of these studies may, in part, be explained by the composition of the patient population and the information provided to them: Clinical trial participants will have been provided high-quality information curated by healthcare professionals with an interest in LS screening, and likely represent a more engaged patient population. This suggests that patient education is likely to have a major impact on perceptions and uptake of screening.

Barriers of finance, logistics, and education may be addressed by government investment in genetics healthcare. Not only do such investments build clinical infrastructure and sequencing capacity, from which dedicated LS screening pipelines will benefit, they also popularise genetics. The 100,000 Genomes Project (100kGP) has had a significant impact on genetics services in the UK. Initial challenges faced by the 100kGP included pipelines for DNA extraction, establishing quick turnaround times from DNA sample receipt to analysis, and data storage and access. Whilst the utility of whole-genome sequencing to direct patient care is not agreed by all clinicians, the

100kGP has demonstrated the feasibility of turnaround times <18 days, and has established data management systems that include secure links to patients' clinical records and several national datasets [61]. Large-scale initiatives also provide an opportunity to explore associated ethical, legal, and social issues, such as the impact on different peoples (particularly the clinically underserved), accountability within the clinical pathway, and the patients' privacy, data security, and role as stakeholders [62], all of which will be informative for LS screening programs.

References

1. Vasen, H.; Blanco, I.; Aktan-Collan, K.; Gopie, J.; Alonso, A.; Aretz, S.; Bernstein, I.; Bertario, L.; Burn, J.; Capella, G.; et al. Revised guidelines for the clinical management of Lynch syndrome (HNPCC): Recommendations by a group of European experts. Gut 2013, 62, 812–823.
2. Dominguez-Valentin, M.; Sampson, J.R.; Seppälä, T.T.; Ten Broeke, S.W.; Plazzer, J.-P.; Nakken, S.; Engel, C.; Aretz, S.; Jenkins, M.A.; Sunde, L.; et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: Findings from the Prospective Lynch Syndrome Database. Med. Off. J. Am. Coll. Med. Genet. 2020, 22, 15–25.
3. Le, D.; Durham, J.; Smith, K.; Wang, H.; Bartlett, B.; Aulakh, L.; Lu, S.; Kemberling, H.; Wilt, C.; Luber, B.; et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017, 357, 409–413.
4. Burn, J.; Sheth, H.; Elliott, F.; Reed, L.; Macrae, F.; Mecklin, J.-P.; Möslein, G.; McRonald, F.E.; Bertario, L.; Evans, D.G.; et al. Cancer prevention with aspirin in hereditary colorectal cancer (Lynch syndrome), 10-year follow-up and registry-based 20-year data in the CAPP2 study: A double-blind, randomised, placebo-controlled trial. Lancet 2020, 395, 1855–1863.
5. Cuzick, J.; Thorat, M.A.; Bosetti, C.; Brown, P.H.; Burn, J.; Cook, N.R.; Ford, L.G.; Jacobs, E.J.; Jankowski, J.A.; la Vecchia, C.; et al. Estimates of benefits and harms of prophylactic use of aspirin in the general population. Oncol. Off. J. Eur. Soc. Med. Oncol. 2015, 26, 47–57.
6. Dueñas, N.; Navarro, M.; Teulé, À.; Solanes, A.; Salinas, M.; Iglesias, S.; Munté, E.; Ponce, J.; Guardiola, J.; Kreisler, E.; et al. Assessing effectiveness of colonic and gynecological risk reducing surgery in Lynch syndrome individuals. Cancers 2020, 12, 3419.
7. Win, A.; Jenkins, M.; Dowty, J.; Antoniou, A.; Lee, A.; Giles, G.; Buchanan, D.; Clendenning, M.; Rosty, C.; Ahnen, D.; et al. Prevalence and penetrance of major genes and polygenes for colorectal cancer. Cancer Epidemiol. Biomark. Prev. 2017, 26, 404–412.
8. Moreira, L.; Balaguer, F.; Lindor, N.; de la Chapelle, A.; Hampel, H.; Aaltonen, L.; Hopper, J.; Marchand, L.L.; Gallinger, S.; Newcomb, P.; et al. Identification of Lynch syndrome among patients with colorectal cancer. Jama 2012, 308, 1555–1565.

9. Ryan, N.A.J.; Glaire, M.A.; Blake, D.; Cabrera-Dandy, M.; Evans, D.G.; Crosbie, E.J. The proportion of endometrial cancers associated with Lynch syndrome: A systematic review of the literature and meta-analysis. Med. 2019, 21, 2167–2180.
10. Douglas, J.; Gruber, S.; Meister, K.; Bonner, J.; Watson, P.; Krush, A.; Lynch, H. History and molecular genetics of Lynch syndrome in family G: A century later. JAMA 2005, 294, 2195–2202.
11. Lynch, H.T.; Krush, A.J. Cancer family “G” revisited: 1895–1970. Cancer 1971, 27, 1505–1511.
12. Lynch, H.; Kimberling, W.; Albano, W.; Lynch, J.; Elston, R.; Biscone, K.; Schuelke, G.; Sandberg, A.; Lipkin, M.; Deschner, E.; et al. Hereditary nonpolyposis colorectal cancer (Lynch syndromes I and II). I. Clinical description of resource. Cancer 1985, 56, 934–938.
13. Aaltonen, L.; Peltomäki, P.; Leach, F.; Sistonen, P.; Pylkkänen, L.; Mecklin, J.; Järvinen, H.; Powell, S.; Jen, J.; Hamilton, S.; et al. Clues to the pathogenesis of familial colorectal cancer. Science 1993, 260, 812–816.
14. Lothe, R.; Peltomäki, P.; Meling, G.; Aaltonen, L.; Nyström-Lahti, M.; Pylkkänen, L.; Heimdal, K.; Andersen, T.; Møller, P.; Rognum, T.; et al. Genomic instability in colorectal cancer: Relationship to clinicopathological variables and family history. Cancer Res. 1993, 53, 5849–5852.
15. Thibodeau, S.; Bren, G.; Schaid, D. Microsatellite instability in cancer of the proximal colon. Science 1993, 260, 816–819.
16. Ionov, Y.; Peinado, M.; Malkhosyan, S.; Shibata, D.; Perucho, M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 1993, 363, 558–561.
17. Strand, M.; Prolla, T.; Liskay, R.; Petes, T. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature 1993, 365, 274–276.
18. Fishel, R.; Lescoe, M.; Rao, M.; Copeland, N.; Jenkins, N.; Garber, J.; Kane, M.; Kolodner, R. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993, 75, 1027–1038.
19. Leach, F.S.; Nicolaides, N.C.; Papadopoulos, N.; Liu, B.; Jen, J.; Parsons, R.; Peltomäki, P.; Sistonen, P.; Aaltonen, L.A.; Nyström-Lahti, M.; et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 1993, 75, 1215–1225.
20. Bronner, C.; Baker, S.; Morrison, P.; Warren, G.; Smith, L.; Lescoe, M.; Kane, M.; Earabino, C.; Lipford, J.; Lindblom, A.; et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 1994, 368, 258–261.
21. Nicolaides, N.; Papadopoulos, N.; Liu, B.; Wei, Y.; Carter, K.; Ruben, S.; Rosen, C.; Haseltine, W.; Fleischmann, R.; Fraser, C.; et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 1994, 371, 75–80.

22. Miyaki, M.; Konishi, M.; Tanaka, K.; Kikuchi-Yanoshita, R.; Muraoka, M.; Yasuno, M.; Igari, T.; Koike, M.; Chiba, M.; Mori, T. Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Genet. 1997, 17, 271–272.
23. Ligtenberg, M.; Kuiper, R.; Chan, T.; Goossens, M.; Hebeda, K.; Voorendt, M.; Lee, T.; Bodmer, D.; Hoenselaar, E.; Hendriks-Cornelissen, S.; et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3′ exons of TACSTD1. Genet. 2009, 41, 112–117.
24. Herman, J.; Umar, A.; Polyak, K.; Graff, J.; Ahuja, N.; Issa, J.; Markowitz, S.; Willson, J.; Hamilton, S.; Kinzler, K.; et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Natl. Acad. Sci. USA 1998, 95, 6870–6875.
25. Deng, G.; Chen, A.; Hong, J.; Chae, H.; Kim, Y. Methylation of CpG in a small region of the hMLH1 promoter invariably correlates with the absence of gene expression. Cancer Res. 1999, 59, 2029–2033.
26. Boland, C.R. Evolution of the nomenclature for the hereditary colorectal cancer syndromes. Cancer 2005, 4, 211–218.
27. National Institute for Health and Care Excellence UK. Molecular Testing Strategies for Lynch Syndrome in People with Colorectal Cancer [Diagnostic Guidance 27]. Available online: https://www.nice.org.uk/guidance/dg27 (accessed on 21 December 2020).
28. Gylling, A.H.S.; Nieminen, T.T.; Abdel-Rahman, W.M.; Nuorva, K.; Juhola, M.; Joensuu, E.I.; Järvinen, H.J.; Mecklin, J.P.; Aarnio, M.; Peltomäki, P.T. Differential cancer predisposition in Lynch syndrome: Insights from molecular analysis of brain and urinary tract tumors. Carcinogenesis 2008, 29, 1351–1359.
29. Yurgelun, M.; Kulke, M.; Fuchs, C.; Allen, B.; Uno, H.; Hornick, J.; Ukaegbu, C.; Brais, L.; McNamara, P.; Mayer, R.; et al. Cancer susceptibility gene mutations in individuals with colorectal cancer. Clin. Oncol. 2017, 35, 1086–1095.
30. Hampel, H.; Pearlman, R.; Beightol, M.; Zhao, W.; Jones, D.; Frankel, W.; Goodfellow, P.; Yilmaz, A.; Miller, K.; Bacher, J.; et al. Assessment of tumor sequencing as a replacement for lynch syndrome screening and current molecular tests for patients with colorectal cancer. JAMA Oncol. 2018, 4, 806–813.
31. Porkka, N.K.; Olkinuora, A.; Kuopio, T.; Ahtiainen, M.; Eldfors, S.; Almusa, H.; Mecklin, J.-P.; Peltomäki, P. Does breast carcinoma belong to the Lynch syndrome tumor spectrum? Somatic mutational profiles vs. ovarian and colorectal carcinomas. Oncotarget 2020, 11, 1244-1256.
32. Knudson, A.G. Mutation and cancer: Statistical study of retinoblastoma. Natl. Acad. Sci. USA 1971, 68, 820.

33. Domingo, E.; Laiho, P.; Ollikainen, M.; Pinto, M.; Wang, L.; French, A.; Westra, J.; Frebourg, T.; Espín, E.; Armengol, M.; et al. BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. Med. Generics 2004, 41, 664–668.
34. Kambara, T.; Simms, L.; Whitehall, V.; Spring, K.; Wynter, C.; Walsh, M.; Barker, M.; Arnold, S.; McGivern, A.; Matsubara, N.; et al. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. Gut 2004, 53, 1137–1144.
35. Pérez-Carbonell, L.; Alenda, C.; Payá, A.; Castillejo, A.; Barberá, V.; Guillén, C.; Rojas, E.; Acame, N.; Gutiérrez-Aviñó, F.; Castells, A.; et al. Methylation analysis of MLH1 improves the selection of patients for genetic testing in Lynch syndrome. Mol. Diagn. 2010, 12, 498–504.
36. Parsons, M.; Buchanan, D.; Thompson, B.; Young, J.; Spurdle, A. Correlation of tumour BRAF mutations and MLH1 methylation with germline mismatch repair (MMR) gene mutation status: A literature review assessing utility of tumour features for MMR variant classification. Med. Generics 2012, 49, 151–157.
37. Stoffel, E.; Mangu, P.; Gruber, S.; Hamilton, S.; Kalady, M.; Lau, M.; Lu, K.; Roach, N.; Limburg, P. Hereditary colorectal cancer syndromes: American society of clinical oncology clinical practice guideline endorsement of the familial risk-colorectal cancer: European society for medical oncology clinical practice guidelines. Clin. Oncol. 2015, 33, 209–217.
38. Balmana, J.; Balaguer, F.; Cervantes, A.; Arnold, D. Familial risk-colorectal cancer: ESMO Clinical Practice Guidelines. Oncol. 2013, 24, 73–80.
39. Rodriguez-Bigas, M.; Boland, C.; Hamilton, S.; Henson, D.; Jass, J.; Khan, P.; Lynch, H.; Perucho, M.; Smyrk, T.; Sobin, L.; et al. A national cancer institute workshop on hereditary nonpolyposis colorectal cancer syndrome: Meeting highlights and Bethesda guidelines. Natl. Cancer Inst. 1997, 89, 1758–1762.
40. Umar, A.; Boland, C.; Terdiman, J.; Syngal, S.; de la Chapelle, A.; Rüschoff, J.; Fishel, R.; Lindor, N.; Burgart, L.; Hamelin, R.; et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. Natl. Cancer Inst. 2004, 96, 261–268.
41. Mvundura, M.; Grosse, S.; Hampel, H.; Palomaki, G. The cost-effectiveness of genetic testing strategies for Lynch syndrome among newly diagnosed patients with colorectal cancer. Med. 2010, 12, 93–104.
42. Ladabaum, U.; Wang, G.; Terdiman, J.; Blanco, A.; Kuppermann, M.; Boland, C.; Ford, J.; Elkin, E.; Phillips, K. Strategies to identify the Lynch syndrome among patients with colorectal cancer: A cost-effectiveness analysis. Intern. Med. 2011, 155, 69–79.
43. Snowsill, T.; Huxley, N.; Hoyle, M.; Jones-Hughes, T.; Coelho, H.; Cooper, C.; Frayling, I.; Hyde, C. A systematic review and economic evaluation of diagnostic strategies for Lynch syndrome.

Health Technol. Assess. 2014, 18, doi:10.3310/hta18580.

44. Di Marco, M.; Dandrea, E.; Panic, N.; Baccolini, V.; Migliara, G.; Marzuillo, C.; de Vito, C.; Pastorino, R.; Boccia, S.; Villari, P. Which Lynch syndrome screening programs could be implemented in the “real world”? A systematic review of economic evaluations. Med. 2018, 20, 1131–1144.
45. Tognetto, A.; Michelazzo, M.B.; Calabró, G.E.; Unim, B.; di Marco, M.; Ricciardi, W.; Pastorino, R.; Boccia, S. A systematic review on the existing screening pathways for Lynch syndrome identification. Public Health 2017, 5, 243.
46. Heald, B.; Plesec, T.; Liu, X.; Pai, R.; Patil, D.; Moline, J.; Sharp, R.R.; Burke, C.A.; Kalady, M.F.; Church, J.; et al. Implementation of universal microsatellite instability and immunohistochemistry screening for diagnosing Lynch Syndrome in a large academic medical center. Clin. Oncol. 2013, 31, 1336–1340.
47. Shaikh, T.; Handorf, E.; Meyer, J.; Hall, M.; Esnaola, N. Mismatch repair deficiency testing in patients with colorectal cancer and nonadherence to testing guidelines in young adults. JAMA Oncol. 2018, 4, e173580.
48. Hampel, H.; de la Chapelle, A. The search for unaffected individuals with Lynch syndrome: Do the ends justify the means? Cancer Prev. Res. 2011, 4, 1–5.
49. Royal College of Pathologists; Bowel Cancer UK, 2016 Data Briefing: Reflex Testing for Lynch Syndrome in People Diagnosed with Bowel Cancer under the Age of 50. Available online: https://bowelcancerorguk.s3.amazonaws.com/Final2016DataBriefingLynchsyndrome.pdf (accessed on 21 December 2020).
50. Loughrey, M.; Quirke, P.; Shepherd, N. Royal College of Pathologists, Dataset for Colorectal Cancer Histopathology Reports. Available online: https://www.rcpath.org/asset/E94CE4A2-D722-44A7-84B9D68294134CFC/ (accessed on 21 December 2020).
51. Noll, A.; Parekh, P.J.; Zhou, M.; Weber, T.K.M.D.; Ahnen, D.; Ms, X.-C.W.; Karlitz, J.J. Barriers to Lynch syndrome testing and preoperative result availability in earlyonset colorectal cancer: A national physician survey study. Transl. Gastroenterol. 2018, 9, 185.
52. Dicks, E.; Pullman, D.; Kao, K.; MacMillan, A.; Simmonds, C.; Etchegary, H. Universal tumor screening for Lynch syndrome: Perspectives of Canadian pathologists and genetic counselors. Community Genet. 2019, 10, 335–344.
53. Brennan, B.; Hemmings, C.T.; Clark, I.; Yip, D.; Fadia, M.; Taupin, D.R. Universal molecular screening does not effectively detect Lynch syndrome in clinical practice. Adv. Gastroenterol. 2017, 10, 361–371.
54. Kidambi, T.D.; Lee, R.; Terdiman, J.P.; Day, L. Successful implementation of Lynch syndrome screening in a safety net institution. Community Genet. 2016, 7, 255–260.

55. Muller, C.; Lee, S.M.; Barge, W.; Siddique, S.M.; Berera, S.; Wideroff, G.; Tondon, R.; Chang, J.; Peterson, M.; Stoll, J.; et al. Low referral rate for genetic testing in racially and ethnically diverse patients despite universal colorectal cancer screening. Gastroenterol. Hepatol. 2018, 16, 1911–1918.
56. Bombard, Y.; Rozmovits, L.; Sorvari, A.; Daly, C.; Carroll, J.C.; Kennedy, E.; Rabeneck, L.; Baxter, N.N. Universal tumor screening for Lynch syndrome: Health-care providers' perspectives. Med. 2017, 19, 568–574.
57. Smith, S.; Foy, R.; McGowan, J.; Kobayashi, L.; Burn, J.; Brown, K.; Side, L.; Cuzick, J. General practitioner attitudes towards prescribing aspirin to carriers of Lynch syndrome: Findings from a national survey. Cancer 2017, 16, 509–516.
58. Melissa, K.F.; Mollie, A.B.; Michael, J.W.; Jolyn, S.T.; Stephanie, N.L.; Kevin, H. Lynch syndrome: Awareness among medical students at a United States Medical School. Women's Health Rev. 2012, 8, 242–247.
59. Tan, Y.Y.; Spurdle, A.B.; Obermair, A. Knowledge, attitudes and referral patterns of lynch syndrome: A survey of clinicians in australia. Pers. Med. 2014, 4, 218–244.
60. Hunter, J.E.; Zepp, J.M.; Gilmore, M.J.; Davis, J.V.; Esterberg, E.J.; Muessig, K.R.; Peterson, S.K.; Syngal, S.; Acheson, L.S.; Wiesner, G.L.; et al. Universal tumor screening for Lynch syndrome: Assessment of the perspectives of patients with colorectal cancer regarding benefits and barriers. Cancer 2015, 121, 3281–3289.
61. Turnbull, C. Introducing whole-genome sequencing into routine cancer care: The Genomics England 100,000 Genomes Project. Oncol. Off. J. Eur. Soc. Med. Oncol. 2018, 29, 784–787.
62. Sankar, P.L.; Parker, L.S. The precision medicine initiative's all of us research program: An agenda for research on its ethical, legal, and social issues. Med. Off. J. Am. Coll. Med. Genet. 2017, 19, 743–750.