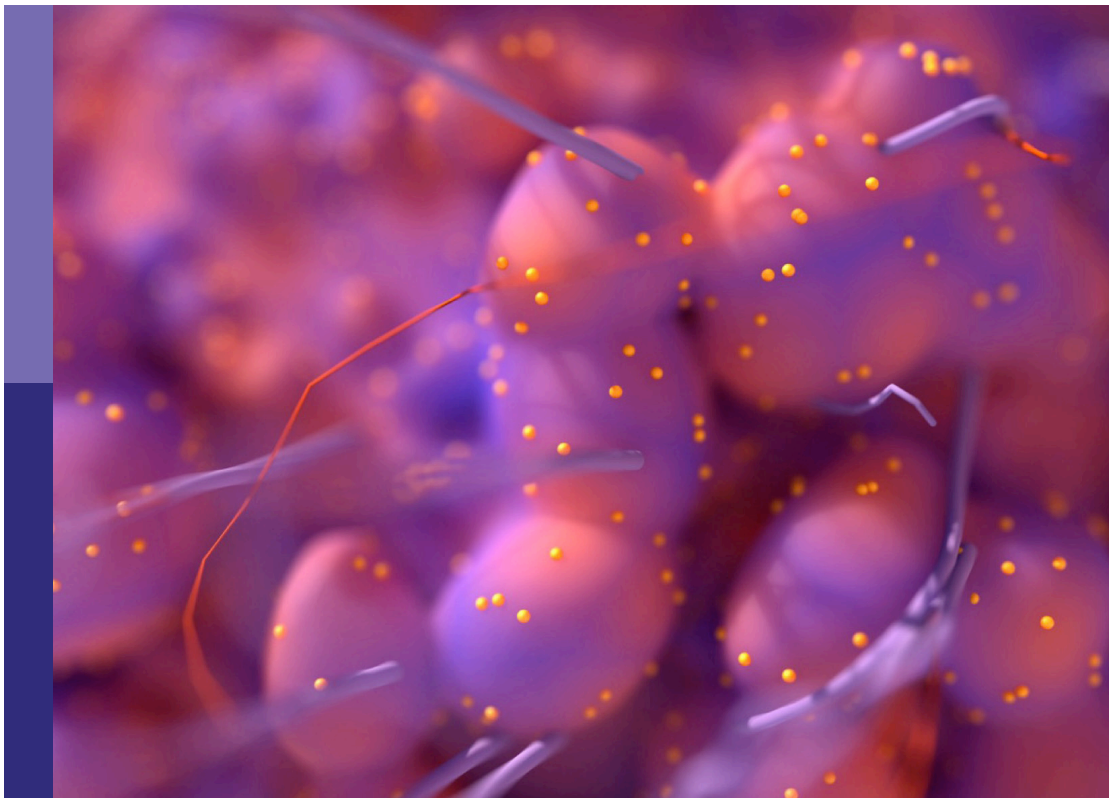
frontiers | Research Topics

Identification, risk stratification, and optimized management for lynch syndrome

Edited by
Inge Bernstein, Christina Therkildsen and Toni T. Seppälä

Published in
Frontiers in Oncology



FRONTIERS EBOOK COPYRIGHT STATEMENT

The copyright in the text of individual articles in this ebook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this ebook is the property of Frontiers.

Each article within this ebook, and the ebook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this ebook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or ebook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-8325-2785-6
DOI 10.3389/978-2-8325-2785-6

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Identification, risk stratification, and optimized management for lynch syndrome

Topic editors

Inge Bernstein — Aalborg University Hospital, Denmark

Christina Therkildsen — Copenhagen University Hospital, Denmark

Toni T. Seppälä — Helsinki University Central Hospital, Finland

Citation

Bernstein, I., Therkildsen, C., Seppälä, T. T., eds. (2023). *Identification, risk stratification, and optimized management for lynch syndrome*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2785-6

Table of contents

frontiers | Frontiers in Oncology

TYPE Editorial
PUBLISHED 08 June 2023
DOI 10.3389/fonc.2023.1223568

Check for updates

OPEN ACCESS

EDITED AND REVIEWED BY
Claudio Sette,
Catholic University of the Sacred Heart, Rome, Italy

*CORRESPONDENCE
Inge Bernstein
i.bernstein@rn.dk
Christina Therkildsen
Christina.Therkildsen@regionh.dk
Toni T. Seppälä
toni.seppala@helsinki.fi

RECEIVED 16 May 2023
ACCEPTED 23 May 2023
PUBLISHED 08 June 2023

CITATION
Bernstein I, Therkildsen C and Seppälä TT (2023) Editorial: Identification, risk stratification, and optimized management for Lynch Syndrome.
Front. Oncol. 13:1223568.
doi: 10.3389/fonc.2023.1223568

COPYRIGHT
© 2023 Bernstein, Therkildsen and Seppälä. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Editorial: Identification, risk stratification, and optimized management for Lynch Syndrome

Inge Bernstein[1,2*], Christina Therkildsen[3*] and Toni T. Seppälä[4*]

[1]Department of Surgical Gastroenterology, Aalborg University Hospital, Aalborg, Denmark, [2]Department of Clinical Medicine, Aalborg University, Aalborg, Denmark, [3]Gastro Unit, Copenhagen University Hospital, Hvidovre, Denmark, [4]Faculty of Medicine and Health Technology, Tampere University and TAYS Cancer Centre, Tampere, Finland

KEYWORDS

Lynch Syndrome, mismatch repair genes, identification, surveillance, personalized medicine

Editorial on the Research Topic
Identification, risk stratification, and optimized management for Lynch Syndrome

Introduction

Lynch Syndrome (LS) is caused by a pathogenic variant in one of the mismatch repair (MMR) genes, leading to cancers in various organs, including the colorectum (CRC), endometrium (EC), ovaries (OVC), stomach, small bowel, biliary tract, pancreas, and urinary tract (UTC). For decades, there has been a focus on defining, diagnosing, and treating LS, particularly since the discovery of the first MMR genes responsible for LS in the early 90s. Now, a new era is underway, focusing on personalized medicine.

This Research Topic aims to explore potential approaches to improve the identification of LS patients, technologies for individualized cancer risk estimation to guide more personalized cancer treatment and surveillance programs, and issues focusing on the quality improvement of established surveillance programs.

To achieve these goals, invitations to submit papers were sent through the Frontiers platform and via email to well-established colleagues in the LS research community. Thirteen articles — four reviews, seven original papers, and two case reports — were accepted for publication after review, all addressing various objectives within the scope.

Identification and overall clinical aspects

Historically, research on LS has focused on CRC, followed later by EC. Well-established surveillance programs for these cancer types have been implemented, improving prognosis. Consequently, cancer-related deaths in LS patients are more frequently caused by other extracolonic LS-associated cancers (1).

In this Research Topic, Zalevskaja et al. demonstrated improved survival after pancreaticobiliary cancers in LS compared to sporadic cancer, although prognosis was still poor. Pancreatic and biliary tract cancers are rare tumour types among LS carriers, and Zalevskaja et al. call for more studies on molecular and immune profiles to learn about LS-associated pancreaticobiliary cancers' suitability to possible immunotherapy.

Williams et al. present the landscape of current practice and future perspectives in the clinical management of LS patients in a minireview, touching on LS diagnostics, risk estimates, surveillance strategies around the world, and treatment options.

Strategies for identifying LS patients have evolved over time, from using clinical Amsterdam and Bethesda criteria towards guidelines recommending testing all newly identified CRCs for deficient MMR (dMMR) by MMR protein immunohistochemistry (IHC) or by microsatellite instability (MSI). Recently, recommendations have been extended to include all endometrial cancers and other extracolonic cancers known in LS. Cost-effectiveness analyses vary in different studies and are often country-specific. However, due to enhanced detection of LS and prognostic implications, universal testing of adenocarcinomas in several tumour types is recommended by numerous professional societies, including the National Comprehensive Cancer Network (NCCN), Society of Gynecologic Oncology (SGO), ACOG, and European Society for Medical Oncology (ESMO).

Tumour analysis as an initial screening tool for LS identification

Concordance between such screening methods can vary for different tumour types. This variation is not necessarily important in universal testing of newly diagnosed cancers to identify LS, but caution should be taken when using abnormal IHC-guided single-gene genetic testing in CRC, EC, and OVC to identify LS patients, as this method may miss 8% of patients with LS according to Pan et al..

Moreover, a new potential screening tool is presented in this Research Topic, which may be relevant for screening of extracolonic cancers. Rasmussen et al. demonstrated that LS-associated UTC frequently exhibits loss of MMR protein expression, and they found no significant differences between IHC and a sequencing-based MSI approach using 54 markers.

Risk stratification

Traditionally, carriers of any pathogenic variant in an MMR gene were thought to have a comparable risk of developing a range of different malignancies without distinction to the affected gene. Establishment of international LS databases, such as the Prospective Lynch Syndrome Database (PLSD) and the International Mismatch Repair Consortium (IMRC) database, and several other studies have improved the understanding that cumulative incidences of cancers and survival in LS-associated cancer patients are associated with the specific gene affected (1–3). The differences in risk may be explained by molecular variation in the driver mutations (4, 5). Even within each MMR gene or a specific variant, the cancer risk can still vary from <10 to >80% suggesting unknown genetic, epigenetic or environmental risk factors (3).

The review by Andini et al address the mild phenotype of *PMS2* carriers, specifically pointing out the low risk of CRCs that paradoxically may have more in common, biologically, with sporadic CRC as behaving more aggressive with a worse prognosis than LS-CRC induced by defects in *MLH1*, *MSH2* or *MSH6*.

LS is the most common cause of inherited CRC, but for women with LS, EC is most likely the sentinel cancer unless surgically prevented. Underkofler and Ring suggest, based on recently updated literature on identification and management of gynecologic cancers in LS, that gynecologists should be allowed to identify and prevent such cancers to improve prognosis in light of treatment changes towards more molecular classification and targeted therapy.

The use of molecular analysis to classify EC have also important clinical implication according to Riedinger et al (2023), who demonstrated that EC with epigenetic MMR defects have a poorer outcome that is independently associated with lymph node metastases. They therefore suggest implementation of MMR status and hypermethylation preoperatively for risk stratification of the EC patients.

Colorectal cancer surveillance and outcomes

The adenoma-to-carcinoma sequence has traditionally been considered the pathway to CRC, leading to the recommendation of colonoscopy and polypectomy as optimal cancer prevention methods in international guidelines. However, the appropriate length of surveillance intervals and the degree of individualization in tailoring programs remain debated.

Approximately 6% of Lynch Syndrome (LS) patients undergoing colonoscopy surveillance exhibit multiple adenomas. Jain et al.show that the majority (87%) of these patients had advanced neoplasia or CRC, indicating that multiple adenomas represent a high-risk phenotype independent of genotype. This finding suggests that the presence of multiple adenomas should be considered when developing recommendations for individualized CRC surveillance.

Jamizadeh et al. also supports personalized surveillance intervals that take individual risk factors into account. Their findings showed that 35% of surveillance-detected CRC cases were found after 24 months, and thus outside their recommended biennial surveillance program. Additional factors, such as *MLH1* and *MSH2* pathogenic variants, male sex, current or previous smoking status, and high BMI were associated with an elevated risk of developing CRC and might be considered when setting the surveillance intervals.

Carmen et al. did, however, not find any statistically significant differences in the likelihood of developing adenomas, advanced adenomas, or CRC between carriers of variants in the four MMR genes. However, they observed a higher or earlier incidence of adenomas in carriers of *MSH2/EPCAM* variants and a non-significant tendency of increased risk among Hispanics.

Consequently, they advocated for individualized screening programs and further research.

Many authors of this Research Topic emphasize the need for future prospective studies and, based on their findings, more individualized guidelines for LS management.

New treatment approaches

Immune checkpoint-based therapy (ICT) has proven effective in managing solid microsatellite instability-high (MSI-H) tumours, regardless of organ site. LS patients may be optimal candidates for ICT, as most cancers in LS exhibit deficient mismatch repair (dMMR), MSI, and immune response activation. Though molecular differences have been observed between sporadic and LS MSI cancers, a systematic literature review found no difference in response rates between LS and sporadic MSI cancer patients (6).

Atiq et al. presented a case report of an LS patient with locally advanced prostate cancer who achieved significant tumour reduction after treatment with ICT in combination with androgen deprivation. Another case report by Liu et al. described an LS patient with adenocarcinoma in the rectum and prostate, followed by undifferentiated sarcoma in the neck, who achieved tumour regression and stable disease after ICT in combination with chemotherapy.

It is important to emphasize that data are still scarce, and the majority of studies presenting positive treatment response may indicate a selection bias towards publication, particularly in case reports. Therefore, more studies, preferentially from large clinical trials, are needed to evaluate the outcome of ICT in LS.

Prevention by vaccination

The new era of precision oncology, based on tumour molecular profiling to tailor personalized treatment and immune system modulation, has transformed cancer prevention approaches for at-risk individuals. MMR deficiency in LS carriers leads to the accumulation of mutations in coding microsatellites, giving rise to frameshift peptides (FSP) recognized by the immune system as neoantigens. Cancer vaccines composed of commonly recurring FSP neoantigens have been evaluated in mouse models (7).

In a comprehensive review, Sei et al. (2023) discuss advances, technologies, and prevention strategies for the clinical translation of personalized risk-tailored vaccination strategies for LS carriers.

Perspectives of the future

Despite the improved management of LS carriers in recent decades, there is still a need for research on studies addressing cancer development, prevention, and geographical differences to improve the prognosis for LS patients.

In summary, this Research Topic offers a comprehensive overview of the current state of LS research, highlighting recent advances and future directions in identification, surveillance, and treatment. The growing emphasis on personalized medicine holds promise for more effective management of LS patients, ultimately leading to improved outcomes and quality of life for those affected by this genetic syndrome.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Dominguez-Valentin M, Haupt S, Seppälä TT, Sampson JR, Sunde L, Bernstein I, et al. Mortality by age, gene and gender in carriers of pathogenic mismatch repair gene variants receiving surveillance for early cancer diagnosis and treatment: a report from the prospective lynch syndrome database. *eClinicalMedicine* (2023) 58. doi: 10.1016/j.eclinm.2023.101909

2. Suerik M, Rodríguez-Girondo M, van der Klift HM, Colas C, Brugieres L, Lavoine N, et al. An alternative approach to establishing unbiased colore. *Genet Med* (2019) 21(12):2706–12. doi: 10.1038/s41436-019-0577-z

3. Win AK, Dowty JG, Reece JC, Lee G, Templeton AS, Plazzer JP, et al. Variation in the risk of colorectal cancer in families with lynch syndrome: a retrospective cohort study. *Lancet Oncol* (2021) 22(7):1014–22. doi: 10.1016/S1470-2045(21)00189-3

4. Ahadova A, Stenzinger A, Seppälä TT, Robert Hüneburg R, Kloor M, Bläker H, et al. "Two-in-One hit" model of shortcut carcinogenesis in *MLH1* lynch syndrome carriers. *Gastroentorol* (2023). doi: 10.1053/j.gastro.2023.03.007

5. Helderman CN, Van Der WERF A, Morreau H, Boot A, Van Wezel T, Nielsen M, et al. Molecular profile of *MSH6*-associated colorectal carcinomas shows distinct features from other lynch syndrome–associated colorectal carcinomas. *Gastroentorol* (2023) 1–4. doi: 10.1053/j.gastro.2023.03.198

6. Therkildsen, Jensen LH, Rasmussen M, Bernstein I. An update on immune checkpoint therapy for the treatment of lynch syndrome. *Clin Exp Gastroenterol* (2021) 14:181–97. doi: 10.2147/CEG.S278054

7. Gebert J, Gelincik O, Oezcan-Wahlbrink M, Marshall JD, Hernandez-Sanchez A, Urban K, et al. Recurrent frameshift neoantigen vaccine elicits protective immunity with reduced tumor burden and improved overall survival in a lynch syndrome mouse model. *Gastroenterology* (2021) 161(4):1288–1302.e13. doi: 10.1053/j.gastro.2021.06.073

frontiers | Frontiers in Oncology

TYPE Case Report
PUBLISHED 15 November 2022
DOI 10.3389/fonc.2022.1014859

Check for updates

OPEN ACCESS

EDITED BY
Christina Therkildsen,
Copenhagen University Hospital,
Denmark

REVIEWED BY
Haiping Jiang,
Zhejiang University, China
Lin Dong,
Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, China
Yibo Gao,
Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, China

*CORRESPONDENCE
Xiu Nie
niexiuyishi@126.com
Gang Peng
penggang1977@aliyun.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 09 August 2022
ACCEPTED 26 October 2022
PUBLISHED 15 November 2022

CITATION
Liu J, Chang X, Xiao G, Zhong J, Huang B, Zhang J, Gao B, Peng G and Nie X (2022) Case report: Undifferentiated sarcoma with multiple tumors involved in Lynch syndrome: Unexpected favorable outcome to sintilimab combined with chemotherapy.
Front. Oncol. 12:1014859.
doi: 10.3389/fonc.2022.1014859

COPYRIGHT
© 2022 Liu, Chang, Xiao, Zhong, Huang, Zhang, Gao, Peng and Nie. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Case report: Undifferentiated sarcoma with multiple tumors involved in Lynch syndrome: Unexpected favorable outcome to sintilimab combined with chemotherapy

Jiaying Liu[1†], Xiaona Chang[1†], Guixiang Xiao[1], Jingmin Zhong[1], Bo Huang[1], Jiwei Zhang[1], Beibei Gao[1], Gang Peng[2*] and Xiu Nie[1*]

[1]Department of Pathology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2]Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: Patients with Lynch syndrome are at an increased risk of developing simultaneous or metachronous tumors, while sarcomas have been occasionally reported. Sarcomas are generally not considered part of the common Lynch syndrome tumor spectrum. However, more and more studies and case reports suggested that sarcoma could be a rare clinical manifestation of Lynch syndrome, leading to new treatment strategies for sarcoma.

Case summary: We report the case of a 74-year-old male patient with Lynch syndrome who had rectal mucinous adenocarcinoma and prostate adenocarcinoma and then developed undifferentiated sarcoma of the left neck two years later. Mismatch repair deficiency (dMMR) was confirmed by immunohistochemical staining for the mismatch repair proteins MSH2, MSH6, MLH1 and PMS2. The result of polymerase chain reaction (PCR) microsatellite instability (MSI) testing of sarcoma showed high-level microsatellite instability (MSI-H). Additionally, a pathogenic germline mutation in MSH2 (c.2459-12A>G) was detected by next-generation sequencing (NGS). Taking into account HE morphology, immunohistochemical phenotype, MSI status, NGS result, medical history and germline MSH2 gene mutation, the pathological diagnosis of left neck biopsy tissue was Lynch syndrome related undifferentiated sarcoma with epithelioid morphology. The patient has been receiving immunotherapy (sintilimab) combined with chemotherapy (tegafur, gimeracil and oteracil potassium capsules) and currently has stable disease. We also reviewed the literature to understand the association between sarcoma and Lynch syndrome.

Conclusion: Sarcoma may now be considered a rare clinical manifestation of Lynch syndrome. Attention and awareness about the association between

Lynch syndrome and sarcoma need to be increased. Therefore, timely detection of MMR proteins and validation at the gene level for suspicious patients are the keys to avoiding missed or delayed diagnosis and to identifying patients suited for immunotherapy, which may also help to provide appropriate genetic counseling and follow-up management for patients.

KEYWORDS

undifferentiated sarcoma, immune checkpoint inhibitor, sintilimab, lynch syndrome, MSH2, mismatch repair deficiency

Introduction

Lynch syndrome is a hereditary cancer predisposition syndrome caused by a germline mutation in one of several DNA mismatch repair (MMR) genes (including MLH1, MSH2, MSH6, PMS2) or loss of expression of MSH2 due to deletion in the EPCAM gene (1, 2). Individuals with Lynch syndrome are at an increased risk of developing simultaneous or metachronous tumors, predominantly colorectal cancer and endometrial cancer (3, 4), and are also at increased risk of cancer of the ovary, prostate, stomach, genitourinary system, and hepatobiliary system (2). Moreover, sarcomas are generally not considered part of the common Lynch syndrome tumor spectrum. However, patients with Lynch syndrome have been occasionally reported to develop sarcomas (5–11). As more and more studies and case reports published, the opinion that sarcoma could be a rare clinical manifestation of Lynch syndrome is getting more and more attention, leading to new treatment strategies for sarcoma.

We reported a case in which undifferentiated sarcoma of the neck was identified two years later in a patient with Lynch syndrome who had rectal mucinous adenocarcinoma and prostate adenocarcinoma. The patient has been receiving immunotherapy (sintilimab) combined with chemotherapy (tegafur, gimeracil and oteracil potassium capsules) and currently has stable disease. Furthermore, we also reviewed the literature to understand the association between sarcoma and Lynch syndrome. The report aims to raise awareness of Lynch syndrome-related sarcomas and to identify patients suited for immunotherapy.

Case presentation

We present the case according to the CARE reporting checklist (Supplementary Figure S1; available at https://www.care-statement.org/checklist).

A 74-year-old male patient with left neck swelling for one month, without tenderness, and without fever or other symptoms was admitted to Union Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology in July 2021. A CT-scan of the neck showed a 66 mm×54 mm round soft tissue mass shadow in the left neck (Figure 1A), supraclavicular area and superior mediastinum, with multiple enlarged lymph nodes around, and the trachea, thyroid and esophagus were pushed to the right, with local tracheal narrowing. Further contrast-enhanced CT scans showed ring enhancement (Figure 1B), and a lack of clear demarcation between the mass and the esophageal wall. In addition, tumor markers were normal.

It is noteworthy that the patient was diagnosed with rectal mucinous adenocarcinoma 30 months ago and subsequently underwent surgery. And there was no special treatment after surgery. Meanwhile, the patient was diagnosed with prostate adenocarcinoma on biopsy (Gleason 3 + 4 = 7) and then received castration therapy. The patient reported no family history of tumors.

More specifically, the surgical pathology confirmed a diagnosis of rectal mucinous adenocarcinoma (Figure 1E), while the tumor invaded through the muscularis propria and into the adipose tissue outside the intestinal wall (pT3), without tumor vascular thrombus or perineural invasion around the tumor, with negative surgical margins. There was no evidence of lymph node involvement (14 lymph nodes were resected), while there was one peri-intestinal cancer nodule. Mismatch repair deficiency (dMMR) was confirmed by immunohistochemical staining for the mismatch repair proteins MSH2, MSH6, MLH1 and PMS2. Immunohistochemistry showed complete loss of MSH2 and MSH6 expression but normal MLH1 and PMS2 expression (Figure 2). Germline MSH2 gene mutation (c.2459-12A>G) was detected by next generation sequencing (NGS). NGS result of MSH2 also showed that tumor tissues had higher mutation abundance than the control (84% vs 48%, respectively) (Supplementary Figure S2). This genetic variant was located

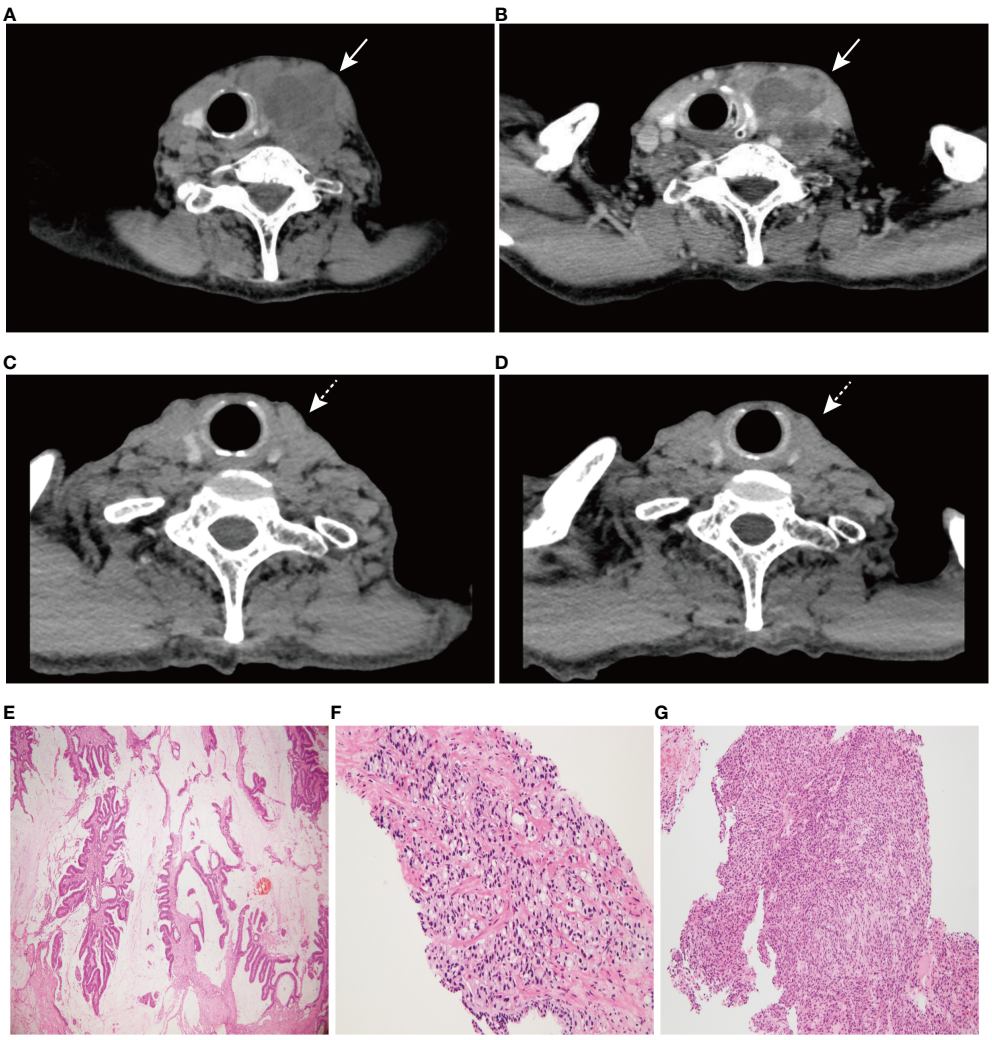


FIGURE 1
CT scan images of neck and hematoxylin-eosin (HE) staining of three tumors (×100). **(A)** A 66 mm×54 mm round soft tissue mass shadow in the left neck. **(B)** Further contrast-enhanced CT scan showed ring enhancement. **(C)** CT scan image of neck at 6 months post-treatment. **(D)** CT scan image of neck at 10 months post-treatment. **(E)** HE staining of rectal mucinous adenocarcinoma, **(F)** prostate adenocarcinoma, and **(G)** undifferentiated sarcoma of the left neck. Solid arrows indicate tumor masses; dashed arrows indicate significant tumor regression.

within an intron and did not generally affect the function of protein. It was not represented in the large population databases (1000 Genomes, gnomAD, and ExAC), indicating that this mutation was a rare variant. In addition, the ClinVar database contained six records for the variant, where the pathogenicity was recorded as likely pathogenic of three records and uncertain significance of three remaining records (12–16). In summary, MSH2 gene mutation (c.2459-12A>G) was classified as likely pathogenic according to American College of Medical Genetics and Genomics guidelines (ACMG, 2015). Moreover, gene mutations strongly associated with treatment and prognosis in rectal cancer were also detected by NGS, including KRAS (p. G12D), PIK3CA (p. E545G) and TP53 (p. R248W). Moreover, NGS test of rectal mucinous adenocarcinoma indicated high TMB (68.79 mutations/Mb) and mutations in other mismatch repair related genes (ATM, CDK12, FANCA and MRE11).

The pathological diagnosis of prostate biopsy revealed prostate adenocarcinoma with Gleason 3 + 4 (Figure 1F). Similarly, dMMR was also confirmed by immunohistochemical staining. Immunohistochemistry showed complete loss of MSH2 and MSH6 expression but normal MLH1 and PMS2 expression (Figure 2). The deficiency of mismatch repair function has several important consequences, such as gain of growth advantage, increase in the point mutation rate, MSI-H and abnormal MMR

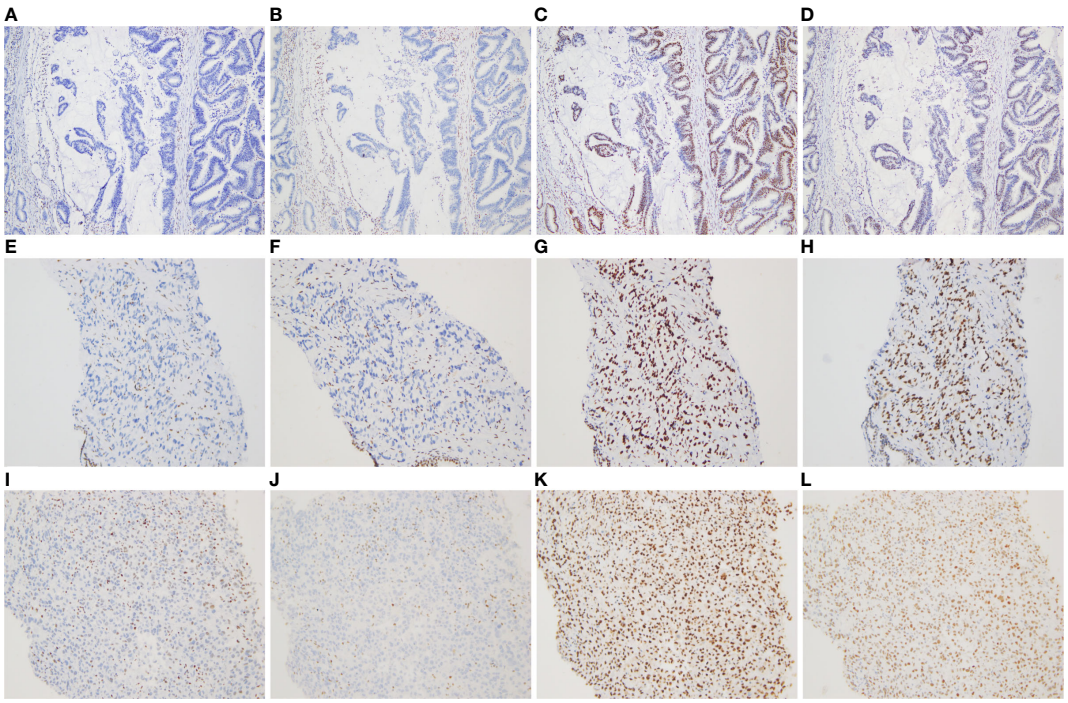


FIGURE 2
Immunohistochemical staining for DNA mismatch repair proteins (MMR proteins MSH6, MSH2, MLH1, and PMS2) of three tumors (x100). **(A–D)** Absence of MSH6 and MSH2 staining, and positive staining for MLH1 and PMS2 in the rectal mucinous adenocarcinoma; **(E–H)** Absence of MSH6 and MSH2 staining, and positive staining for MLH1 and PMS2 in the prostate adenocarcinoma; **(I–L)** Absence of MSH6 and MSH2 staining, and positive staining for MLH1 and PMS2 in the undifferentiated sarcoma of left neck.

protein expression by IHC. In summary, we reached consensus that the prostate adenocarcinoma and rectal mucinous adenocarcinoma were associated with Lynch syndrome.

After comprehensive consideration of the patient's history and status, the patient underwent biopsy of the left neck mass. Microscopically, tumor cells displayed striking atypia and epithelioid morphology, infiltrating into skeletal muscle, without lymph node structure detected (Figure 1G). In addition, the lack of differentiation of the immunohistochemical phenotype led to difficulty in understanding the tumor cell of origin. For more details, see Supplementary Table 1. Furthermore, NGS, including genes and mutations associated with soft tissue sarcoma typing (57 genes, 236 types of gene fusions and 14 gene mutations), was performed. TP53 mutation (p. Arg175His) was detected, but we were still unable to determine the tumor cell of origin. As expected, dMMR was also confirmed by immunohistochemical staining, consistent with the phenotypes of rectal mucinous adenocarcinoma and prostate adenocarcinoma (Figure 2). In addition, the result of polymerase chain reaction (PCR) microsatellite instability (MSI) testing of sarcoma showed high-level microsatellite instability (MSI-H). For more details, see Supplementary Figure S3.

In summary, taking into account HE morphology, immunohistochemical phenotype, NGS result, MSI result, medical history and germline MSH2 gene mutation (c.2459-12A>G), the pathological diagnosis of left neck biopsy tissue was Lynch syndrome-related undifferentiated sarcoma with epithelioid morphology.

Among the differential diagnoses, the diagnosis of metastatic cancer of the left neck was ruled out due to a lack of expression of epithelial markers (PCK, CK8/18, CK7, CK20, Villin, CDX2, PSAP, etc.). Lack of expression of malignant melanoma markers (S100, SOX10, HMB45, MelanA, etc.) made it impossible to make the diagnosis of malignant melanoma. Similarly, the absence of detection of lymphatic and hematopoietic system markers (LCA, CD3, CD20, CD38, CD138, MUM1, Kappa, Lambda, MPO, CD43, CD117, etc.) was unable to support the diagnosis of lymphatic and hematopoietic cancer. In addition, the diagnosis of sarcoma with certain differentiation was hard to make due to the absence of lineage-specific markers (Desmin, ERG, CD34, or corresponding fusion genes and mutant genes). In addition, it was unreasonable to make a diagnosis of sporadic undifferentiated sarcoma, which barely demonstrated immunohistochemical absence of MMR proteins and had no pathogenic or likely pathogenic germline gene mutations.

The patient has been receiving 15 cycles of immunotherapy (sintilimab, 200 mg i.v. every three weeks) combined with oral chemotherapy (tegafur, gimeracil and oteracil potassium capsules) and well tolerated. Reassuringly, significant regression of the left neck tumor was observed after two cycles of treatment, and the curative effect was evaluated as partial response (PR) according to the RECIST criteria and then maintained the state of PR during a follow-up of 14 months, which further supported our diagnosis (Figures 1C, D). The timeline scheme of the major clinical event of the patient is represented in Figure 3.

Discussion

We present a case report of a male patient who was diagnosed with rectal mucinous adenocarcinoma and prostate adenocarcinoma at age 71 and left neck undifferentiated sarcoma at age 74. Immunohistochemical staining for MMR proteins of three tumors yielded consistent results, MSH6 (–), MSH2 (-), MLH1 (+), and PMS2 (+), indicating the presence of dMMR. In addition, the result of PCR MSI testing of sarcoma showed MSI-H. Moreover, the patient carries a germline likely pathogenic MSH2 gene mutation (c.2459-12A>G). All things considered, the final pathological diagnosis of the left neck tumor was Lynch syndrome-related undifferentiated sarcoma with epithelioid morphology.

Sarcoma is a rare clinical manifestation of Lynch syndrome (8, 17, 18). We summarized sarcomas reported in conjunction with Lynch syndrome (except for occasional cases reported in non-English literature) in Table 1. A previous study in the Prospective Lynch Syndrome Database showed an increase in the incidence and lifetime risk of sarcoma, although details of specific illness risk and mutated genes were not reported (17). The study enrolled 6,350 patients with Lynch syndrome, and 16 of them developed sarcomas (12 osteosarcomas and 4 soft tissue sarcomas) after 51,646 follow-up years (17), which meant that patients with Lynch syndrome had more than 50-fold and 1.2-fold higher incidence of osteosarcomas and soft tissue sarcomas compared with the expected rates in the general population (osteosarcomas 0.34 per 100,000, soft tissue sarcomas 5.03 per 100,000), respectively (37–39). An Asian study demonstrated tumor development in 55 Japanese Lynch syndrome patients and reported a patient developing sarcoma with germline MLH1 mutation (8). Recently, a cohort study by de Angelis et al. evaluated the occurrence of sarcomas in a cohort of patients with tumors on the Lynch syndrome spectrum and finally identified five eligible cases, three of which carried MSH2 pathogenic variants (18).

Some previous studies indicated that the development of sarcoma in patients with Lynch syndrome was associated with the expression of MMR proteins, thereby connecting sarcoma with MMR genes (5, 7, 9–11, 28, 33). Furthermore, previous studies have shown that MMR genes may be associated with sarcoma risk (18, 40–42). A study of cancer susceptibility variants based on The Cancer Genome Atlas (TCGA) data described that two MSH2 mutation carriers were detected in an unselected sarcoma population (225 patients) and classified MSH2 as potentially associated with sarcoma risk according to variant burden analysis (odds ratio, 9.9; $p = 0.02$; false discovery rate, 0.09) (40). In addition, Mirabello et al. analyzed pathogenic germline variants in cancer-susceptibility genes in 1244 patients with osteosarcoma and found more germline MSH2 pathogenic variants in patients with osteosarcoma than in the control group ($p < 0.05$) (41). Moreover, a previous study showed that sarcoma tended to be more associated with pathogenic variants of MSH2 than other MMR genes, as 25 of 43 (58.1%) tested cases had MSH2 germline mutations (18). It was a significantly higher frequency in patients with sarcoma than in unselected patients with Lynch syndrome, where MSH2 was usually the second most frequently mutated gene (seen in approximately 40% of patients) (17, 43).

With the advent of the era of tumor immunity, immune checkpoint inhibitor therapy has become an effective treatment for microsatellite instability-high (MSI-H) or dMMR tumors (44, 45). Latham A et al. assessed the MSI status of 15,045 patients (more than 50 cancer types) based on NGS data, and the incidence of MSI-H and MSI-indeterminate (MSI-I) in soft

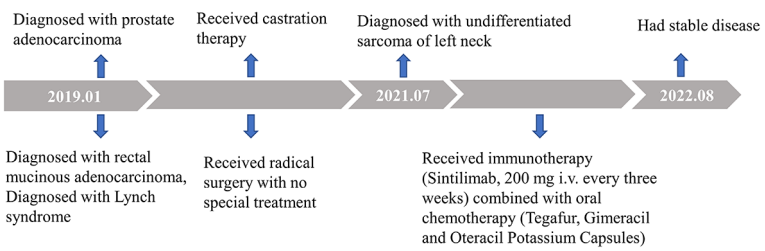


FIGURE 3
Timeline scheme of the major clinical event of the patient.

TABLE 1 Overview of sarcomas linked to Lynch syndrome published in the literature.

Year	Authors	Sarcoma	Expression of MMR proteins	MSI status	Germline MMR gene mutation
2021	Lam SW et al. (11)	Pleomorphic rhabdomyosarcoma	MSH2 and MSH6 loss	NA	MSH2 p. Cys697Tyr
2020	de Angelis de Carvalho N et al. (18)	Soft-tissue sarcoma	MSH2 and MSH6 loss	NA	MSH2 c.1444A>T; p.Arg482Ter-P
		Osteosarcoma	MSH2 and MSH6 loss	MSI-H	MSH2 c.1661+1G>A-LP
		Myxoid Liposarcoma	Intact	NA	MLH1 exon 17 to 19 deletion-P
		Liposarcoma and Osteosarcoma	MSH2 and MSH6 loss	MSI-H	MSH2 c.2152C>T; p.Gln718Ter-P
2019	Doyle L et al. (19)	Pleomorphic rhabdomyosarcoma	MSH2 and MSH6 loss	NA	MSH2 c.2152C>T; p.Gln718Ter-P
2019	Latham A et al. (20)	Soft-tissue sarcoma	MSH2 and MSH6 loss	MSI-I	MSH2 c.1216C>T; p.Arg406Ter
		Soft-tissue sarcoma	MSH2 and MSH6 loss	MSI-I	MSH2 c.229_230delAG; p.Ser77Cysfs*4
		Soft-tissue sarcoma	NA	MSS	PMS2 del exon 8-9
		Soft-tissue sarcoma	NA	MSS	MSH2 c.942+3A>T
2019	Kazmi S et al. (21)	Malignant phyllodes tumor with stromal or sarcomatous overgrowth	MSH6 partially loss	MSS	MSH6 mutation not specified
2019	Björkman P et al. (22)	Angiosarcoma	MLH1 loss	NA	MLH1 mutation not specified
2018	Tlemsani C et al. (23)	Rhabdomyosarcoma	MLH1 and PMS2 loss	MSS	MLH1 c.1863_1864insT; p.Leu622Serfs*10
2018	Saita C et al. (8)	Sarcoma not specified	Intact	NA	MLH1 mutation not specified
2017	Carnevali IW et al. (24)	Ovary carcinosarcoma	MSH6 loss	MSI-H	MSH6 c.931_935delAAAAG; p.Lys311Glufs*4
2016	Nguyen A et al. (25)	Myxofibrosarcoma	MLH1 and PMS2 loss	MSI-H	MLH1 c.678-7_686del16
2015	Schiavi A et al. (26)	Leiomyosarcoma	MSH2 and MSH6 loss	NA	MSH2 c.649dupA; p.Ile217Asnfs*15
		Leiomyosarcoma	NA	NA	MLH1 c.2195_2198dupAACA
2013	Yozu M et al. (27)	Pleomorphic liposarcoma	MSH2 and MSH6 loss	NA	MSH2 mutation not specified
2012	Urso E et al. (28)	Leiomyosarcoma	MSH2 and MSH6 loss	MSS	MSH2 del exon 1–16
2011	Brieger A et al. (5)	Malignant fibrous histiocytoma	MSH2 loss	MSI-H	MSH2 c.2038C>T; p.Arg680Ter
		Malignant fibrous histiocytoma	MSH2 loss	MSI-H	MSH2 c.942+3A>T
2009	Yu VP et al. (29)	Leiomyosarcoma	MLH1 loss	MSI-H	MLH1 c.200G>A; p.Gly67Glu
2009	Nilbert M et al. (30)	Sarcoma not specified	NA	NA	MSH2 c.145_148delGACG; p.Asp49Argfs*14
		Liposarcoma	MSH2 and MSH6 loss	MSS	MSH2 c.942+3A>T
		Sarcoma not specified	NA	NA	MSH2 c.942+3A>T
		Carcinosarcoma	MSH2/MSH6 loss	MSI-H	MSH2 c.1165C>T; p.Arg389Ter
		Gliosarcoma	MSH2 and MSH6 loss	NA	MSH2 c.1696_1697delAAinsG; p.Asn566Valfs*24
		Liposarcoma	MSH2 and MSH6 loss	NA	MSH2 c.1-?_366+?del
		Chondrosarcoma	Intact	NA	MLH1 c.1204A>T; p.Lys402Ter
		Sarcoma not specified	NA	NA	MLH1 c.1204A>T; p.Lys403Ter
		Osteosarcoma	NA	NA	MLH1 c.1276C>T; p.Gln426Ter
		Liposarcoma	NA	NA	MLH1 c.1732+?_c.2268del
		Carcinosarcoma	MSH6 loss	NA	MSH6 c.1085delC; p.Pro362Leufs*9
		Leiomyosarcoma	NA	NA	MSH6 c.3514_3515insA; p.Arg1172Lysfs*5
		Malignant hemangiopericytoma	Intact	NA	MSH6 c.3850_3851insATTA; p.Thr1284Asnfs*6
2008	Geary J et al. (31)	Soft-tissue sarcoma	MLH1 loss	NA	MLH1 mutation not specified
2007	South SA et al. (32)	Carcinosarcoma	MLH1 loss	NA	MLH1 c.1896G>C; p.Glu632Asp
2006	Hirata K et al. (33)	Liposarcoma	MSH2 loss	NA	MSH2 c.677delAT; p.Arg227Glufs*19
2003	Lynch HT et al. (34)	Osteosarcoma	NA	MSI-H	MSH2 exon 4 splice site mutation

(Continued)

TABLE 1 Continued

Year	Authors	Sarcoma	Expression of MMR proteins	MSI status	Germline MMR gene mutation
		Malignant fibrous histiocytoma	NA	MSI-H	MSH2 del exon 3–8
2003	den Bakker MA et al. (35)	Rhabdomyosarcoma	MSH2 loss	MSI-H	MSH2 mutation not specified
2000	Sijmons R et al. (36)	Malignant fibrous histiocytoma	MSH2 loss	MSI-H	MSH2 p.Gly429Ter

NA, not available; MSI-H, high microsatellite instability; MSI-I, indeterminate microsatellite instability; MSS, microsatellite stability.

tissue sarcomas was found to be 5.7% (45/785), while two of them were diagnosed with Lynch syndrome with pathogenic MSH2 variants (20). Similarly, another recent study based on NGS data reported that the incidence of dMMR in an unselected cohort of adult soft tissue and bone sarcomas was 2.3% (7/304) (19). Somatic mutation analysis showed that all seven patients had MMR gene mutations (4 of MSH2 or EPCAM, 2 of PMS2, 1 of MSH6), and further germline sequencing of three patients (2 of MSH2, 1 of MSH6) suggested that one patient had pathogenic MSH2 germline mutation and was also diagnosed with Lynch syndrome (19). Tlemsani C et al. highlighted the importance of identifying Lynch syndrome in patients with sarcoma (23). The article described a 19-year-old male patient who presented with metastatic chemoresistant pleomorphic rhabdomyosarcoma. Then, the patient received anti- programmed death (PD)-1 antibody therapy (nivolumab) due to detection of the MLH1 germline pathogenic variant and achieved a rapid complete response of the lung metastases, which appeared sustained after a 1-year follow-up (23). Furthermore, data from the phase II KEYNOTE-158 study of pembrolizumab (an anti-PD-1 monoclonal antibody) in patients with previously treated, advanced noncolorectal MSI-H/dMMR cancer (including 14 sarcomas) demonstrated the clinical benefit of anti-PD-1 therapy among patients with sarcoma (46).

In the present case, undifferentiated sarcoma of the left neck was identified two years later in a 74-year-old male patient with Lynch syndrome who had rectal mucinous adenocarcinoma and prostate adenocarcinoma. The conventional chemotherapy drugs for undifferentiated sarcoma were adriamycin, ifosfamide, gemcitabine, paclitaxel, etc (47–49). The patient refused all intravenous chemotherapy due the older age. Despite the lack of reliable evidence, there were several studies showed that the fluorouracil was effective against undifferentiated sarcoma (50, 51). Therefore, the patient has been receiving immunotherapy (sintilimab) combined with chemotherapy (tegafur, gimeracil and oteracil potassium capsules). Reassuringly, significant regression of the left neck tumor was observed, and the patient was in good condition after a follow-up of 14 months.

In conclusion, sarcoma may now be considered a rare clinical manifestation of Lynch syndrome. Although the risk of sarcoma was significantly lower than that of other common Lynch syndrome-associated tumors, attention to and awareness of the association between Lynch syndrome and sarcoma need to be increased. Therefore, timely detection of MMR proteins by IHC and validation at the gene level for suspicious patients are the keys to avoiding missed or delayed diagnosis and to identifying patients suited for immunotherapy, which may also help to provide appropriate genetic counseling and follow-up management for patients.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Conception/Design: XN and XC; Provision of study material or patients: GP, GX and JinZ; Collection and/or assembly of data: XC and JL; Data analysis and interpretation: JL; Manuscript writing: JL and XC; Final approval of manuscript: GP and XN. All authors have read and approved the submitted version of the manuscript.

Funding

The research was supported by Union Hospital, Tongji Medical College, Huazhong University of Science and Technology Research Fund. (2021xhyn080).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.1014859/full#supplementary-material

References

1. Ligtenberg MJ, Kuiper RP, Geurts van Kessel A, Hoogerbrugge N. Epcam deletion carriers constitute a unique subgroup of lynch syndrome patients. *Fam Cancer* (2013) 12(2):169–74. doi: 10.1007/s10689-012-9591-x

2. Liccardo R, De Rosa M, Izzo P, Duraturo F. Novel implications in molecular diagnosis of lynch syndrome. *Gastroenterol Res Pract* (2017) 2017:2595098. doi: 10.1155/2017/2595098

3. Lee HS, Kim WH, Kwak Y, Koh J, Bae JM, Kim KM, et al. Molecular testing for gastrointestinal cancer. *J Pathol Transl Med* (2017) 51(2):103–21. doi: 10.4132/jptm.2017.01.24

4. Zhao S, Chen L, Zang Y, Liu W, Liu S, Teng F, et al. Endometrial cancer in lynch syndrome. *Int J Cancer* (2022) 150(1):7–17. doi: 10.1002/ijc.33763

5. Brieger A, Engels K, Schaefer D, Plotz G, Zeuzem S, Raedle J, et al. Malignant fibrous histiocytoma is a rare lynch syndrome-associated tumor in two German families. *Familial Cancer* (2011) 10(3):591–5. doi: 10.1007/s10689-011-9455-9

6. Yozu M, Symmans P, Dray M, Griffin J, Han C, Ng D, et al. Muir–Torre syndrome-associated pleomorphic liposarcoma arising in a previous radiation field. *Virchows Archiv* (2013) 462(3):355–60. doi: 10.1007/s00428-012-1369-x

7. Lee N, Luthra R, Lopez-Terrada D, Wang WL, Lazar AJ. Retroperitoneal undifferentiated pleomorphic sarcoma having microsatellite instability associated with Muir-torre syndrome: Case report and review of literature. *J Cutan Pathol* (2013) 40(8):730–3. doi: 10.1111/cup.12172

8. Saita C, Yamaguchi T, Horiguchi SI, Yamada R, Takao M, Iijima T, et al. Tumor development in Japanese patients with lynch syndrome. *PloS One* (2018) 13(4):e0195572. doi: 10.1371/journal.pone.0195572

9. Shigematsu Y, Yamashita K, Takamatsu M, Tanizawa T, Togashi Y, Nakajima T, et al. Primary intramucosal synovial sarcoma of the sigmoid colon in a patient with a germline mutation in the Msh2 gene: A case report. *Pathol Int* (2020) 70(12):1015–9. doi: 10.1111/pin.13020

10. Liu Y, GuLiBaHa M, Yue YB, Li MW, Cao SB, Yan M. An isolated childhood myeloid sarcoma with germline Msh6 mutation-a case report. *Transl Pediatr* (2021) 10(8):2136–43. doi: 10.21037/tp-21-326

11. Lam SW, Kostine M, de Miranda N, Schoffski P, Lee CJ, Morreau H, et al. Mismatch repair deficiency is rare in bone and soft tissue tumors. *Histopathology* (2021) 79(4):509–20. doi: 10.1111/his.14377

12. Mangold E, Pagenstecher C, Friedl W, Fischer HP, Merkelbach-Bruse S, Ohlendorf M, et al. Tumours from Msh2 mutation carriers show loss of Msh2 expression but many tumours from Mlh1 mutation carriers exhibit weak positive Mlh1 staining. *J Pathol* (2005) 207(4):385–95. doi: 10.1002/path.1858

13. Shirts BH, Konnick EQ, Upham S, Walsh T, Ranola JMO, Jacobson AL, et al. Using somatic mutations from tumors to classify variants in mismatch repair genes. *Am J Hum Genet* (2018) 103(1):19–29. doi: 10.1016/j.ajhg.2018.05.001

14. Mangold E, Pagenstecher C, Friedl W, Mathiak M, Buettner R, Engel C, et al. Spectrum and frequencies of mutations in Msh2 and Mlh1 identified in 1,721 German families suspected of hereditary nonpolyposis colorectal cancer. *Int J Cancer* (2005) 116(5):692–702. doi: 10.1002/ijc.20863

15. Barrow E, Jagger E, Brierley J, Wallace A, Evans G, Hill J, et al. Semiquantitative assessment of immunohistochemistry for mismatch repair proteins in lynch syndrome. *Histopathology* (2010) 56(3):331–44. doi: 10.1111/j.1365-2559.2010.03485.x

16. Pope BJ, Clendenning M, Rosty C, Mahmood K, Georgeson P, Joo JE, et al. Germline and tumor sequencing as a diagnostic tool to resolve suspected lynch syndrome. *J Mol Diagn* (2021) 23(3):358–71. doi: 10.1016/j.jmoldx.2020.12.003

17. Dominguez-Valentin M, Sampson JR, Seppala TT, Ten Broeke SW, Plazzer JP, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: Findings from the prospective lynch syndrome database. *Genet Med* (2020) 22(1):15–25. doi: 10.1038/s41436-019-0596-9

18. de Angelis de Carvalho N, Niitsuma BN, Kozak VN, Costa FD, de Macedo MP, Kupper BEC, et al. Clinical and molecular assessment of patients with lynch syndrome and sarcomas underpinning the association with Msh2 germline pathogenic variants. *Cancers (Basel)* (2020) 12(7):1848. doi: 10.3390/cancers12071848

19. Doyle LA, Nowak JA, Nathenson MJ, Thornton K, Wagner AJ, Johnson JM, et al. Characteristics of mismatch repair deficiency in sarcomas. *Modern Pathol* (2019) 32(7):977–87. doi: 10.1038/s41379-019-0202-3

20. Latham A, Srinivasan P, Kemel Y, Shia J, Bandlamudi C, Mandelker D, et al. Microsatellite instability is associated with the presence of lynch syndrome pan-cancer. *J Clin Oncol Off J Am Soc Clin Oncol* (2019) 37(4):286–95. doi: 10.1200/jco.18.00283

21. Kazmi S, Wagner S, Heintzelman R, Corbman M. Malignant phyllodes tumor in lynch syndrome: A case report. *J Med Case Rep* (2019) 13(1):216. doi: 10.1186/s13256-019-2138-0

22. Björkman P, Kantonen I, Blomqvist C, Venermo M, Albäck A. En bloc resection of visceral aorta and right kidney due to aortic sarcoma using temporary extracorporeal bypass grafting. *J Vasc Surg cases innovative techniques* (2019) 5(4):589–92. doi: 10.1016/j.jvscit.2019.08.002

23. Tlemsani C, Leroy K, Gimenez-Roqueplo A-P, Mansuet-Lupo A, Pasmant E, Larousserie F, et al. Chemoresistant pleomorphic rhabdomyosarcoma: Whole exome sequencing reveals underlying cancer predisposition and therapeutic options. *J Med Genet* (2020) 57(2):104–8. doi: 10.1136/jmedgenet-2018-105594

24. Carnevali IW, Cimetti L, Sahnane N, Libera L, Cavallero A, Formenti G, et al. Two cases of carcinosarcomas of the ovary involved in hereditary cancer syndromes. *Int J gynecol Pathol Off J Int Soc Gynecol Pathol* (2017) 36(1):64–70. doi: 10.1097/pgp.0000000000000290

25. Nguyen A, Bougeard G, Koob M, Chenard MP, Schneider A, Maugard C, et al. Msi detection and its pitfalls in cmmrd syndrome in a family with a bi-allelic Mlh1 mutation. *Fam Cancer* (2016) 15(4):571–7. doi: 10.1007/s10689-016-9894-4

26. Schiavi A, Lavigne J, Turcotte R, Kasprzak L, Dumas N, Chong G, et al. Using a family history questionnaire to identify adult patients with increased genetic risk for sarcoma. *Curr Oncol (Toronto Ont)* (2015) 22(5):317–25. doi: 10.3747/co.22.2588

27. Yozu M, Symmans P, Dray M, Griffin J, Han C, Ng D, et al. Muir-Torre syndrome-associated pleomorphic liposarcoma arising in a previous radiation field. *Virchows Archiv an Int J Pathol* (2013) 462(3):355–60. doi: 10.1007/s00428-012-1369-x

28. Urso E, Agostini M, Pucciarelli S, Bedin C, D'Angelo E, Mescoli C, et al. Soft tissue sarcoma and the hereditary non-polyposis colorectal cancer (Hnpcc) syndrome: Formulation of an hypothesis. *Mol Biol Rep* (2012) 39(10):9307–10. doi: 10.1007/s11033-012-1729-2

29. Yu VP, Novelli M, Payne SJ, Fisher S, Barnetson RA, Frayling IM, et al. Unusual presentation of lynch syndrome. *Hereditary Cancer Clin Pract* (2009) 7 (1):12. doi: 10.1186/1897-4287-7-12

30. Nilbert M, Therkildsen C, Nissen A, Akerman M, Bernstein I. Sarcomas associated with hereditary nonpolyposis colorectal cancer: Broad anatomical and morphological spectrum. *Fam Cancer* (2009) 8(3):209–13. doi: 10.1007/s10689-008-9230-8

31. Geary J, Sasieni P, Houlston R, Izatt L, Eeles R, Payne SJ, et al. Gene-related cancer spectrum in families with hereditary non-polyposis colorectal cancer (Hnpcc). *Fam Cancer* (2008) 7(2):163–72. doi: 10.1007/s10689-007-9164-6

32. South SA, Hutton M, Farrell C, Mhawech-Fauceglia P, Rodabaugh KJ. Uterine carcinosarcoma associated with hereditary nonpolyposis colorectal cancer. *Obstet gynecol* (2007) 110(2 Pt 2):543–5. doi: 10.1097/01.Aog.0000275262.60526.01

33. Hirata K, Kanemitsu S, Nakayama Y, Nagata N, Itoh H, Ohnishi H, et al. A novel germline mutation of Msh2 in a hereditary nonpolyposis colorectal cancer patient with liposarcoma. *Am J Gastroenterol* (2006) 101(1):193–6. doi: 10.1111/j.1572-0241.2005.00308.x

34. Lynch HT, Deters CA, Hogg D, Lynch JF, Kinarsky Y, Gatalica Z. Familial sarcoma: Challenging pedigrees. *Cancer* (2003) 98(9):1947–57. doi: 10.1002/cncr.11743

35. den Bakker MA, Seynaeve C, Kliffen M, Dinjens WN. Microsatellite instability in a pleomorphic rhabdomyosarcoma in a patient with hereditary non-polyposis colorectal cancer. *Histopathology* (2003) 43(3):297–9. doi: 10.1046/j.1365-2559.2003.01681.x

36. Sijmons R, Hofstra R, Hollema H, Mensink R, van der Hout A, Hoekstra H, et al. Inclusion of malignant fibrous histiocytoma in the tumour spectrum associated with hereditary non-polyposis colorectal cancer. *Genes Chromosomes Cancer* (2000) 29(4):353–5. doi: 10.1002/1098-2264(2000)9999:9999<::aid-gcc1042>3.0.co;2-t

37. Mirabello L, Troisi RJ, Savage SA. International osteosarcoma incidence patterns in children and adolescents, middle ages and elderly persons. *Int J Cancer* (2009) 125(1):229–34. doi: 10.1002/ijc.24320

38. Misaghi A, Goldin A, Awad M, Kulidjian AA. Osteosarcoma: A comprehensive review. *SICOT J* (2018) 4:12. doi: 10.1051/sicotj/2017028

39. Toro JR, Travis LB, Wu HJ, Zhu K, Fletcher CD, Devesa SS. Incidence patterns of soft tissue sarcomas, regardless of primary site, in the surveillance, epidemiology and end results program, 1978-2001: An analysis of 26,758 cases. *Int J Cancer* (2006) 119(12):2922–30. doi: 10.1002/ijc.22239

40. Huang KL, Mashl RJ, Wu Y, Ritter DI, Wang J, Oh C, et al. Pathogenic germline variants in 10,389 adult cancers. *Cell* (2018) 173(2):355–70 e14. doi: 10.1016/j.cell.2018.03.039

41. Mirabello L, Zhu B, Koster R, Karlins E, Dean M, Yeager M, et al. Frequency of pathogenic germline variants in cancer-susceptibility genes in patients with osteosarcoma. *JAMA Oncol* (2020) 6(5):724–34. doi: 10.1001/jamaoncol.2020.0197

42. Chan SH, Lim WK, Ishak NDB, Li ST, Goh WL, Tan GS, et al. Germline mutations in cancer predisposition genes are frequent in sporadic sarcomas. *Sci Rep* (2017) 7(1):10660. doi: 10.1038/s41598-017-10333-x

43. Vaccaro CA, López-Kostner F, Adriana DV, Palmero EI, Rossi BM, Antelo M, et al. From colorectal cancer pattern to the characterization of individuals at risk: Picture for genetic research in Latin America. *Int J Cancer* (2019) 145(2):318–26. doi: 10.1002/ijc.31920

44. Lee V, Murphy A, Le DT, Diaz LAJr. Mismatch repair deficiency and response to immune checkpoint blockade. *oncol* (2016) 21(10):1200–11. doi: 10.1634/theoncologist.2016-0046

45. Fanale D, Corsini LR, Scalia R, Brando C, Cucinella A, Madonia G, et al. Can the tumor-agnostic evaluation of Msi/Mmr status be the common denominator for the immunotherapy treatment of patients with several solid tumors? *Crit Rev oncology/hematol* (2022) 170:103597. doi: 10.1016/j.critrevonc.2022.103597

46. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite Instability/Mismatch repair-deficient cancer: Results from the phase ii keynote-158 study. *J Clin Oncol Off J Am Soc Clin Oncol* (2020) 38(1):1–10. doi: 10.1200/jco.19.02105

47. Lange SS, Novetsky AP, Powell MA. Recent advances in the treatment of sarcomas in gynecology. *Discovery Med* (2014) 18(98):133–40.

48. Goy BW, Syed S, Padmanabhan A, Burchette RJ, Helmstedter CS. The role of ifosfamide-doxorubicin chemotherapy in histology-specific, high grade, locally advanced soft tissue sarcoma, a 14-year experience. *Radiother Oncol J Eur Soc Ther Radiol Oncol* (2021) 165:174–8. doi: 10.1016/j.radonc.2021.10.019

49. Novetsky AP, Powell MA. Management of sarcomas of the uterus. *Curr Opin Oncol* (2013) 25(5):546–52. doi: 10.1097/CCO.0b013e328363e0ef

50. Lobo-Sanahuja F, Garcia I, Carranza A, Camacho A. Treatment and outcome of undifferentiated carcinoma of the nasopharynx in childhood: A 13-year experience. *Med Pediatr Oncol* (1986) 14(1):6–11. doi: 10.1002/mpo.2950140103

51. Perilongo G, Carli M, Sainati L, Cecchetto G, Mancini A, Cordero di Montezemolo L, et al. Undifferentiated (Embryonal) sarcoma of the liver in childhood: Results of a retrospective Italian study. *Tumori* (1987) 73(3):213–7. doi: 10.1177/030089168707300302

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 20 December 2022
DOI 10.3389/fonc.2022.1038678

Check for updates

OPEN ACCESS

EDITED BY
Toni T. Seppälä,
Helsinki University Central Hospital, Finland

REVIEWED BY
Dimitri Krizzuk,
Aurelia Hospital, Italy
Elizabeth Half,
Rambam Health Care Campus, Israel

*CORRESPONDENCE
Peter P. Stanich
peter.stanich@osumc.edu

SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 07 September 2022
ACCEPTED 30 November 2022
PUBLISHED 20 December 2022

CITATION
Jain A, Alimirah M, Hampel H, Pearlman R, Ma J, Peng J, Kalady MF and Stanich PP (2022) Multiple colorectal adenomas in Lynch syndrome.
Front. Oncol. 12:1038678.
doi: 10.3389/fonc.2022.1038678

COPYRIGHT
© 2022 Jain, Alimirah, Hampel, Pearlman, Ma, Peng, Kalady and Stanich. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Multiple colorectal adenomas in Lynch syndrome

Ayushi Jain[1], Maryam Alimirah[2], Heather Hampel[3], Rachel Pearlman[4], Jianing Ma[5], Jing Peng[5], Matthew F. Kalady[4,6] and Peter P. Stanich[2]*

[1]Department of Internal Medicine, The Ohio State University Wexner Medical Center, Columbus, OH, United States, [2]Division of Gastroenterology, Hepatology and Nutrition, The Ohio State University Wexner Medical Center, Columbus, OH, United States, [3]Division of Clinical Cancer Genomics, City of Hope National Medical Center, Duarte, CA, United States, [4]Division of Human Genetics, The Ohio State University Wexner Medical Center, Columbus, OH, United States, [5]Center for Biostatistics, Department of Biomedical Informatics, The Ohio State University Wexner Medical Center, Columbus, OH, United States, [6]Division of Colorectal Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, United States

Background: Lynch syndrome has not traditionally been considered to have a high colorectal adenoma burden. However, with increasing adenoma detection rates in the general population, the incidence of adenoma detection in Lynch syndrome may also be increasing and leading to higher cumulative adenoma counts.

Aim: To clarify the prevalence and clinical impact of multiple colorectal adenomas (MCRA) in Lynch syndrome.

Methods: A retrospective review of patients with Lynch syndrome at our institution was performed to assess for MCRA (defined as ≥10 cumulative adenomas).

Results: There were 222 patients with Lynch syndrome among whom 14 (6.3%) met MCRA criteria. These patients had increased incidence of advanced neoplasia (OR 10, 95% CI: 2.7-66.7).

Conclusions: MCRA is not unusual in Lynch syndrome and is associated with a significantly increased likelihood of advanced colon neoplasia. Consideration should be given to differentiating colonoscopy intervals based on the presence of polyposis in Lynch syndrome.

KEYWORDS

lynch syndrome, adenomas, colon cancer, multiple adenomas, Lynch

Introduction

Inherited colorectal cancer syndromes are often categorized into "polyposis" and "nonpolyposis" syndromes (1). Lynch syndrome is a hereditary cancer syndrome characterized by heterozygous pathogenic variants in *MLH1*, *MSH2*, *MSH6*, or *PMS2* or an *EPCAM* deletion. Existing literature in Lynch syndrome initially suggested the majority of patients carry *MLH1* or *MSH2* pathogenic variants, although analysis of multigene panel testing results suggest that the frequency is likely similar across the genes and analysis of international registries predicts that *MSH6* and *PMS2* pathogenic variants may be more common (2, 3). Lynch syndrome has classically been associated with a lower colorectal adenoma burden and considered a nonpolyposis syndrome (4, 5). There is limited data on the adenoma burden in patients with Lynch syndrome, though one previous estimate suggested a mean of 7 adenomas by age 80 (6). Another study reported 4% of patients with Lynch syndrome had ≥ 10 cumulative lifetime adenomas and qualified as having a clinical oligopolyposis syndrome, contrary to the traditional assumption that patients with Lynch syndrome do not meet this criteria (7). In addition, with recent reports of nationwide increases in adenoma detection rates, the cumulative lifetime adenoma counts in the Lynch syndrome population are likely increasing along with the general population (8). As such, Lynch syndrome should also be considered in the differential of patients meeting multiple colorectal adenomas criteria, defined as 10 or more adenomas (9).

Lynch syndrome patients have long been recommended to have a colonoscopy every 1-2 years in the United States (1, 10). The latest guidelines from the Mallorca group are now recommending colonoscopy surveillance intervals of 2 to 3 years for most genotypes and up to 5 years for those with *PMS2* mutations (11). However, recent studies have shown that patients with ≥ 10 cumulative lifetime adenomas are at higher risk of advanced neoplasia and colorectal cancer (12). It is unclear if Lynch syndrome patients that meet this criterion would also have additional increased risks.

Our aim was to assess the prevalence of multiple colorectal adenomas in Lynch syndrome and assess for an association with advanced colorectal neoplasia and colorectal cancer.

Methods

This was a retrospective study assessing patients with Lynch syndrome followed in the Hereditary and High-Risk Gastroenterology Clinic. Institutional Review Board approval was obtained prior to study initiation.

Inclusion criteria for the study were age 18 years or greater, a documented pathogenic or likely pathogenic variant in a mismatch repair gene (*MLH1, MSH2, MSH6, PMS2)* or in *EPCAM* on germline genetic testing, the completion of at least one colonoscopy at our institution and a clinic visit from August 2014 through December 2020. Exclusion criteria included a known pathogenic or likely pathogenic variant in additional hereditary cancer genes and a history of total colectomy prior to identification of first adenomatous polyp. All available colonoscopy and pathology records were reviewed to identify polyp characteristics. Colonoscopies were performed with the available endoscopic technology at the time of completion, which included both standard definition and high-definition white light endoscopy and virtual chromoendoscopy. Dye chromoendoscopy was not utilized.

The primary study outcome was the prevalence of multiple colorectal adenomas, defined as ≥ 10 lifetime tubular adenomas (9). The secondary outcomes of interest included prevalence of previous advanced colorectal neoplasia and colorectal cancer in those with and without MCRA. Advanced colorectal neoplasia was defined as a lifetime history of colorectal cancer, advanced adenoma, or advanced sessile serrated lesion. Advanced adenomas were defined as adenomas ≥ 10 mm in size, villous or tubulovillous adenomas or adenomas with high-grade dysplasia. Advanced sessile serrated lesions were defined as sessile serrated polyps ≥ 10 mm in size or with features of high-grade or low-grade dysplasia.

Statistical analysis included univariable analysis to compare patients with 0-9 tubular adenomas and ≥ 10 tubular adenomas. Odds ratio and 95% confidence interval was reported using univariable logistic regression for all variables. Multivariable analysis was utilized to adjust for age.

Results

Two hundred and twenty-two patients met study criteria and were included in the analysis with demographic and clinical details available in Table 1. There were 142 patients (64%) with adenomas in the cohort and 14 patients (6.3%) met criteria for MCRA with 10 or more cumulative adenomatous polyps. The patients with MCRA had a mean of 7 colonoscopies available for review but the majority of patients with MCRA required 3 or less procedures to reach this criterion (13/14, 92.9%). The highest MCRA count was 28 adenomas. The MCRA patients had a mean age of 62 and the two most common mutated genes were *MSH6* (8/14) and *MSH2* (4/14). Of note, 12 (86%) had a history of advanced neoplasia including 5 (36%) with colorectal cancer.

Univariable analysis was performed to compare the cohorts with 0-9 MCRA and ≥ 10 MCRA. Patients with ≥ 10 MCRA were older with a mean age of 62 as compared to 47 in patients with 0-9 MCRA (OR: 1.09, 95% CI: 1.04, 1.15), otherwise the cohorts had similar demographics including similar rates when compared within mismatch repair gene cohorts (Table 2). Patients with ≥ 10 MCRA were significantly more likely to have a history of advanced colorectal neoplasia as compared to patients with only 0-9 MCRA (OR: 10.2, CI: 2.69, 66.7). Notably, this remained true on multivariable analysis adjusted for age (OR: 5.35, CI: 1.34, 35.88).

TABLE 1 Characteristics of patients with Lynch syndrome.

Characteristic	N = 222
Age (Mean, SD)	48.03 (13.36)
Sex	
Female	151 (68%)
Male	71 (32%)
Race	
African American	3 (1.5%)
Asian	4 (2%)
Caucasian	212 (95%)
Hispanic	1 (0.5%)
Other	2 (1%)
Gene	
MLH1	40 (18%)
MSH2	58 (26%)
MSH6	75 (34%)
PMS2	49 (22%)
BMI (log)	3.35 (0.24)
Daily Aspirin	100 (45%)
Colectomy	
None	180 (81%)
Partial	39 (18%)
Total	2 (1%)
Any Cancer	102 (46%)
Colorectal Cancer	41 (18%)
Family History of CRC	164 (76%)
FDR with CRC	98 (46%)
SDR with CRC	128 (59%)
Total Polyps	
0 – 9	187 (84%)
≥ 10	35 (16%)
Adenomas	
0 – 9	208 (93.7%)
≥ 10	14 (6.3%)
Sessile Serrated Adenoma	
None	173 (78%)
≥ 1	49 (22%)
Advanced Neoplasia	89 (40%)

BMI, body mass index; CRC, colorectal cancer, FDR, first degree relative; SDR, second degree relative.

Patients with ≥ 10 MCRA were also more likely to have a personal history of malignancy (OR: 3.12, CI: 1.01, 11.7), although there was not a detected significant difference in a history of colorectal cancer.

Discussion

Lynch syndrome has traditionally been defined as a non-polyposis syndrome and differentiated from polyposis syndromes through a lower adenoma burden (4). In this study, we found that 6% of patients with Lynch syndrome had 10 or more cumulative adenomatous polyps and met MCRA criteria. This is similar to rates of MCRA seen in the general population in a recent study by Sullivan et al. (12). Although this is still a minority of patients with Lynch syndrome, these findings are notable as it is likely that the prevalence of colorectal adenomas in Lynch syndrome will continue to rise in conjunction with increases in adenoma detection rates in the general population. Given this, the mismatch repair genes should be included in multigene panel testing conducted for patients with MCRA as reflected in recent guidelines (13).

Our analysis also revealed that 86% of patients with Lynch syndrome and MCRA had a history of advanced neoplasia and that this was significantly higher than Lynch syndrome patients without polyposis even with adjustment for age. Similarly, Sullivan et al. found that patients without known inherited colorectal syndromes with ≥ 10 MCRA were 17 times more likely to have a history of advanced neoplasia (12). Together, these results indicate that MCRA is a high-risk phenotype for advanced neoplasia independent of genotype and should be taken into consideration to guide individualized recommendations for colorectal cancer surveillance.

A previous international cohort study presented evidence of the existence of unknown familial risk factors that result in wide variations in the risk of colorectal cancer across patients with Lynch syndrome (14). We propose that an MCRA phenotype may be a risk factor for advanced neoplasia and malignancy in patients with Lynch syndrome and should be given consideration when determining surveillance colonoscopy intervals. As guidelines are shifting toward longer colonoscopy intervals, we should consider that patients with a polyposis phenotype independent of Lynch syndrome genotype are likely high risk for advanced neoplasia and malignancy and may benefit from continued close monitoring.

Limitations of our study include the risk of ascertainment bias due to its use of genetic testing results as an inclusion criterion. Additionally, the majority of patients were Caucasian which may limit generalizability across diverse populations. It should also be noted that the majority of our patients carried *MSH6* and *PMS2* variants and thus may not be representative of all Lynch syndrome cohorts. In addition, the use of aspirin may have impacted advanced neoplasia in our patient population and could have a potential confounding effect. Large multi-center and prospective studies are needed to confirm the risks of polyposis in Lynch syndrome and to assess optimal colonoscopy intervals for these patients.

TABLE 2 Univariable analysis of patients with adenomatous oligopolyposis.

Characteristic	0-9 AdenomasN = 208[1]	10+ AdenomasN = 14[1]	OR	95% CI
Gene				
MLH1	39/208 (19%)	1/14 (7.1%)	–	-
MSH2	54/208 (26%)	4/14 (29%)	2.89	0.41, 57.7
MSH6	67/208 (32%)	8/14 (57%)	4.66	0.81, 88.0
PMS2	48/208 (23%)	1/14 (7.1%)	0.81	0.03, 21.0
Age	47.11 (13.05)	61.71 (10.28)	**1.09**	**1.04, 1.15**
Sex				
Female	144/208 (69%)	7/14 (50%)	–	–
Male	64/208 (31%)	7/14 (50%)	2.25	0.74, 6.83
BMI (log)	3.35 (0.24)	3.35 (0.26)	1	0.09, 9.04
Daily Aspirin	96/208 (46%)	4/14 (29%)	0.46	0.49, 1.44.
Race				
Caucasian	200/208 (96%)	12/14 (86%)	-	-
Non-Caucasian	8/208 (3.8%)	2/14 (14%)	4.17	0.59, 19.0
FDR with CRC	90/199 (45%)	8/14 (57%)	1.61	0.54, 5.07
Any Cancer	92/207 (44%)	10/14 (71%)	**3.12**	**1.01, 11.7**
Colorectal Cancer	36/208 (17%)	5/14 (36%)	2.65	0.78, 8.17
Advanced Neoplasia	77/208 (37%)	12/14 (86%)	**10.2**	**2.69, 66.7**

[1] n/N (%); Mean (SD)
OR, Odds Ratio; CI, Confidence Interval; FDR, first degree relative; CRC, colorectal cancer. Bold values indicate statistically significant values.

In summary, MCRA phenotype is not unusual in Lynch syndrome and was associated with a significant increase in history of advanced colon neoplasia. Given this, consideration should be given to individualizing colonoscopy intervals based on the presence of polyposis in Lynch syndrome.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ohio State Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

AJ and PS: conception and design, data collection, drafting of the article, critical revision of the article for important intellectual content, analysis of data, and final approval of the article. MA: data collection, analysis of the data, critical revision of the article for important intellectual content and final approval. JM and JP: Data analysis and final approval. HH, RP, and MK: critical revision of the article for important intellectual content and final approval. All authors contributed to the article and approved the submitted version.

Acknowledgments

Guarantor of the article: PS.

Conflict of interest

PS receives research support from Emtora Biosciences, Janssen Pharmaceuticals Inc., Pfizer Inc. and the PTEN Research Foundation.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW. ACG clinical guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol* (2015) 110(2):223–62. doi: 10.1038/ajg.2014.435

2. Espenschied CR, LaDuca H, Li S, McFarland R, Gau C-L, Hampel H, et al. Multigene panel testing provides a new perspective on lynch syndrome. *J Clin Oncol* (2017) 35(22):2568–75. doi: 10.1200/JCO.2016.71.9260

3. Win AK, Jenkins MA, Dowty JG, Antoniou AC, Lee A, Giles GG, et al. Prevalence and penetrance of major genes and polygenes for colorectal cancer. *Cancer Epidemiol Biomarkers Prev* (2017) 26(3):404–12. doi: 10.1158/1055-9965.EPI-16-0693

4. Lindgren G, Liljegren A, Jaramillo E, Rubio C, Lindblom A. Adenoma prevalence and cancer risk in familial non-polyposis colorectal cancer. *Gut* (2002) 50(2):228–34. doi: 10.1136/gut.50.2.228

5. Idos G, Valle L. Lynch syndrome. In: Adam MP, et al, editors. *GeneReviews* (®). Seattle (WA (1993). University of Washington, Seattle Copyright © 1993-2022, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle, Seattle (WA). All rights reserved.

6. Edelstein DL, Axilbund J, Baxter M, Hylind LM, Romans K, Griffin CA, et al. Rapid development of colorectal neoplasia in patients with lynch syndrome. *Clin Gastroenterol Hepatol* (2011) 9(4):340–3. doi: 10.1016/j.cgh.2010.10.033

7. Kalady MF, Kravochuck SE, Heald B, Burke CA, Church JM. Defining the adenoma burden in lynch syndrome. *Dis Colon Rectum* (2015) 58(4):388–92. doi: 10.1097/DCR.0000000000000333

8. Shaukat A, Holub J, Pike IM, Pochapin M, Greenwald D, Schmitt C, et al. Benchmarking adenoma detection rates for colonoscopy: Results from a US-based registry. *Am J Gastroenterol* (2021) 116(9):1946–9. doi: 10.14309/ajg.0000000000001358

9. Monahan KJ, Bradshaw N, Dolwani S, Desouza B, Dunlop MG, East JE, et al. Guidelines for the management of hereditary colorectal cancer from the British society of gastroenterology (BSG)/Association of coloproctology of great Britain and Ireland (ACPGBI)/United kingdom cancer genetics group (UKCGG). *Gut* (2020) 69(3):411–44. doi: 10.1136/gutjnl-2019-319915

10. Weiss JM, Gupta S, Burke CA, Axell L, Chen L-M, Chung DC, et al. NCCN guidelines® insights: Genetic/Familial high-risk assessment: Colorectal, version 1.2021. *J Natl Compr Canc Netw* (2021) 19(10):1122–32. doi: 10.1164/jnccn.2021.0048

11. Seppälä TT, Latchford A, Negoi I, Sampaio Soares A, Jimenez-Rodriguez R, Sánchez-Guillén L, et al. European Guidelines from the EHTG and ESCP for lynch syndrome: an updated third edition of the mallorca guidelines based on gene and gender. *Br J Surg* (2021) 108(5):484–98. doi: 10.1002/bjs.11902

12. Sullivan BA, Redding TS 4th, Qin X, Gellad ZF, Hauser ER, O'Leary C M, et al. Ten or more cumulative lifetime adenomas are associated with increased risk for advanced neoplasia and colorectal cancer. *Dig Dis Sci* (2021) 6:2526–34. doi: 10.1007/s10620-021-07069-0

13. Heald B, Hampel H, Church J, Dudley B, Hall MJ, Mork ME, et al. Collaborative group of the americas on inherited gastrointestinal cancer position statement on multigene panel testing for patients with colorectal cancer and/or polyposis. *Fam Cancer* (2020) 19(3):223–39. doi: 10.1007/s10689-020-00170-9

14. Consortium. IMR. Variation in the risk of colorectal cancer in families with lynch syndrome: a retrospective cohort study. *Lancet Oncol* (2021) 22(7):1014–22. doi: 10.1016/S1470-2045(21)00189-3

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 30 January 2023
DOI 10.3389/fonc.2023.1069467

Check for updates

OPEN ACCESS

EDITED BY
Inge Bernstein,
Aalborg University Hospital, Denmark

REVIEWED BY
Serena Bonin,
University of Trieste, Italy
Lin Dong,
Chinese Academy of Medical Sciences and Peking Union Medical College, China
Jinglue Song,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Shujuan Pan
span@myriad.com

SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 13 October 2022
ACCEPTED 11 January 2023
PUBLISHED 30 January 2023

CITATION
Pan S, Cox H, Willmott J, Mundt E, Gorringe H, Landon M, Bowles KR, Coffee B, Roa BB and Mancini-DiNardo D (2023) Discordance between germline genetic findings and abnormal tumor immunohistochemistry staining of mismatch repair proteins in individuals with suspected Lynch syndrome.
Front. Oncol. 13:1069467.
doi: 10.3389/fonc.2023.1069467

COPYRIGHT
© 2023 Pan, Cox, Willmott, Mundt, Gorringe, Landon, Bowles, Coffee, Roa and Mancini-DiNardo. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Discordance between germline genetic findings and abnormal tumor immunohistochemistry staining of mismatch repair proteins in individuals with suspected Lynch syndrome

Shujuan Pan*, Hannah Cox, Jamie Willmott, Erin Mundt, Heidi Gorringe, Michelle Landon, Karla R. Bowles, Bradford Coffee, Benjamin B. Roa and Debora Mancini-DiNardo

Myriad Genetic Laboratories, Inc., Salt Lake City, UT, United States

Background and Aims: Tumor immunohistochemical staining (IHC) of DNA mismatch repair (MMR) proteins is often used to guide germline genetic testing and variant classification for patients with suspected Lynch syndrome. This analysis examined the spectrum of germline findings in a cohort of individuals showing abnormal tumor IHC.

Methods: We assessed individuals with reported abnormal IHC findings and referred for testing with a six-gene syndrome-specific panel (n=703). Pathogenic variants (PVs) and variants of uncertain significance (VUS) in MMR genes were designated expected/unexpected relative to IHC results.

Results: The PV positive rate was 23.2% (163/703; 95% confidence interval [CI], 20.1%-26.5%); 8.0% (13/163; 95% CI, 4.3%-13.3%) of PV carriers had a PV in an unexpected MMR gene. Overall, 121 individuals carried VUS in MMR genes expected to be mutated based on IHC results. Based on independent evidence, in 47.1% (57/121; 95% CI, 38.0%-56.4%) of these individuals the VUSs were later reclassified as benign and in 14.0% (17/121; 95% CI, 8.4%-21.5%) of these individuals the VUSs were reclassified as pathogenic.

Conclusions: Among patients with abnormal IHC findings, IHC-guided single-gene genetic testing may miss 8% of individuals with Lynch syndrome. In addition, in patients with VUS identified in MMR genes predicted to be mutated by IHC, extreme caution must be taken when the IHC results are considered in variant classification.

KEYWORDS

hereditary cancer syndrome, clinical genetic testing, cancer diagnosis, universal tumor screening, IHC – immunohistochemistry

Introduction

Lynch syndrome is caused by an inherited germline pathogenic variant (PV) in one or more of the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, and *PMS2* (1, 2). Approximately 3% of colorectal cancer (CRC) cases result from Lynch syndrome, making it the most common heritable CRC syndrome. Lynch syndrome also is associated with endometrial, ovarian, gastric/small bowel, urothelial, central nervous system, pancreatic, and prostate cancers (3). Clinical management for patients with a Lynch syndrome-related cancer involves heightened secondary cancer surveillance and can include risk-reducing surgeries – measures that have been shown to reduce morbidity and mortality (4–6). Therefore, it is essential to distinguish between Lynch syndrome and sporadic disease in patients diagnosed with cancer.

One first-line approach to differentiate between Lynch syndrome and sporadic cancer is to use immunohistochemical (IHC) staining to assess MLH1, MSH2, MSH6, and PMS2 protein expression in tumor tissue from biopsy or surgical resection. The National Comprehensive Cancer Network (NCCN), the American Society for Clinical Oncology, and others recommend universal IHC screening of new CRC and endometrial cancer cases (3, 7). Abnormal tumor MMR protein expression by IHC suggests a deficiency in the corresponding gene(s) and compromised MMR. NCCN recommends referral for further genetic testing for patients with abnormal IHC findings (3, 8–11).

Reflex genetic testing after an abnormal tumor IHC result can follow numerous paths. For example, MLH1 protein expression can be disrupted either by a germline pathogenic variant (PV) in *MLH1*, DNA promoter hypermethylation that silences the gene, or by double somatic mutations. Historically, if MLH1 is absent on IHC for CRC tumors, subsequent testing can take several directions: (1) germline *MLH1* testing; (2) tumor *MLH1* methylation testing; (3) tumor testing for the BRAF p.V600E PV based on its association with *MLH1* methylation status (3, 12). In recent years, tumor testing of the MMR genes to detect somatic mutations in MMR genes has also been recommended (13). Nevertheless, only when gene-specific testing fails to identify a mutation will germline testing of additional MMR genes and/or other genes associated with hereditary cancer syndromes typically be recommended (3). This stepwise approach has proven complex, confusing, and time-consuming. While NCCN guidelines recommend that an individual with expertise in genetics be involved in the diagnostic process (3), surveyed gastroenterologists reported that it is often unclear which specialist would be responsible for selecting and ordering the test (14). Each separate test adds time to the patient's diagnostic journey and increases the risk of loss to follow-up, which can delay or prevent risk-reducing surgical procedures.

Another relevant concern is the sensitivity of MMR IHC, with a 5-10% false negative rate (3, 15). Staining quality can vary depending on the tumor microenvironment and tissue fixation conditions, leading to ambiguity, misinterpretation of results, and misinformed gene selection for testing (16). In addition, some individuals who have abnormal IHC are found to carry germline PVs in MMR genes not predicted by the IHC result (17–19) or in non-MMR genes associated with other cancer syndromes (20, 21), which would have been missed using gene-specific genetics testing guided by IHC results.

In addition to its use as a screening tool, IHC results may be employed as supportive evidence in determining the pathogenicity of variants identified in genes predicted by IHC to be mutated (22). For instance, the variant classification criteria used by the International Society for Gastrointestinal Hereditary Tumors (InSiGHT) indicate that: when the presence of a variant of uncertain significance (VUS) coincides with the absence of the corresponding MMR protein on IHC in two or more patients, it is deemed supporting evidence for class 5 (pathogenic) or class 4 (likely pathogenic); conversely, inconsistent IHC results observed in three or more tumors were considered as supportive evidence for class 2 (likely benign) or class 1 (benign) (23). This application is concerning given the low predictive value of IHC staining for Lynch syndrome (24, 25). Although IHC results generally are not used as stand-alone evidence for variant classification, there exists potential for an incorrect determination (22).

The frequency of discordance between IHC results and germline genetic findings across various tumor types has not been evaluated systematically in the clinical laboratory, meaning that it is unclear how many patients might be affected clinically by incomplete or misleading IHC results. The current analysis aimed to address this knowledge gap by evaluating germline genetic findings from multi-gene panel testing of individuals with abnormal IHC results in Lynch-associated tumor types. The objectives were to determine (1) the extent of PVs in genes not predicted by IHC, and (2) the possibility of misclassifying variants based on IHC findings.

Materials and methods

Patient population

The analysis included individuals who underwent clinical genetic testing that included the MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) from May 2011 through April 2018. Individuals were included if they reported a personal history of cancer (e.g., colorectal, endometrial, ovarian) and/or colorectal polyps and an abnormal MMR IHC test result in a tumor sample type indicated for Lynch syndrome IHC testing (i.e., CRC or endometrial cancer). To eliminate pre-existing mutation bias and ensure the mutation status of all MMR genes were obtained, only patients whose genetic testing included all four genes were assessed. Therefore, the following criteria were not part of our data query: individuals who were tested for a subset of the MMR genes, for ancestry-specific founder mutations, or for a known familial mutation. Testing was performed by Myriad Genetic Laboratories, Inc. (Salt Lake City, UT), a national Clinical Laboratory Improvement Amendments- and College of American Pathology-certified facility. All individuals provided consent for clinical genetic testing, and test data were de-identified and aggregated for analysis. As a retrospective study performed on de-identified samples, this analytical validation was not subject to any additional review (HHS regulation 45 CFR 46 per section § 46.101). Clinical information, including personal history of cancer and the IHC tumor test result, was obtained from the test request form completed by the healthcare provider.

Abbreviations: CRC, colorectal cancer; IHC, immunohistochemistry; LR, large rearrangement; MMR, mismatch repair; PV, pathogenic variant; VUS, variant of uncertain significance.

Genetic testing and variant classification

Genomic DNA was extracted from each patient's blood sample (QIAsymphony; Qiagen, Venlo, The Netherlands), and subjected to genetic testing using a six-gene cancer panel designed for individuals with suspected Lynch syndrome or *MUTYH*-associated polyposis. The panel included *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, and *MUTYH*. Testing included sequencing and large rearrangement analysis of all genes except *EPCAM* (large rearrangement analysis only).

For sequencing analysis, exonic regions and adjacent -20/+10 intron regions of each gene were amplified by Polymerase Chain Reaction (PCR) and sequenced in forward and reverse directions. For *PMS2* exons 11-15 that have high homology to pseudogenes, a long-range PCR was first performed, and the regions of interest were amplified by nested PCR followed by sequencing.

For large rearrangement analysis of *MLH2*, *MSH2*, *MSH6*, *EPCAM* and *MUTYH*, a clinically validated high-density oligonucleotide microarray was used as the primary methodology (26) and multiplex ligation-dependent probe amplification (MLPA) (MRC-Holland, Amsterdam, The Netherlands) was used as confirmatory approach. For large rearrangements in *PMS2*, MLPA was used as the primary methodology. For any copy number changes revealed by MLPA in the pseudogene region of *PMS2*, long-range PCR was also performed to determine whether the large rearrangement was in *PMS2* or the pseudogene.

Variant classification was consistent with guidelines from the American College of Medical Genetics and Genomics, as previously described (27, 28). Variants with a laboratory classification of pathogenic or likely pathogenic were considered PVs. Variants with a laboratory classification of benign or likely benign were considered benign (i.e., clinically insignificant). Variants for which clinical significance could not be determined were classified as VUS.

Analysis

Genetic test results were considered "expected" if a germline PV was detected in an MMR gene consistent with the gene-specific testing strategy recommended by NCCN guidelines for the IHC test result (Supplemental Table 1) (3). For example, detection of a germline *MLH1* or *PMS2* mutation was considered "expected" in an individual who showed loss of MLH1 and/or PMS2 on IHC; however, a germline *MSH2* mutation in this individual would be considered "unexpected". The analysis also partitioned results based on "typical" and "atypical" MMR IHC patterns. In general, typical IHC patterns were those that involved only one of the two characteristic MMR heterodimer pairs, MSH2/MSH6 or MLH1/PMS2, and listed in the NCCN guidelines. Atypical patterns involved MMR proteins from both MMR heterodimer pairs. For proportions, an exact 95% confidence interval (CI) was calculated.

Results

Analysis group characteristics

A total of 703 individuals were included in this analysis. Table 1 shows clinical and demographic characteristics according to the genetic test performed. Overall, CRC and endometrial were the most common cancers for these individuals, with CRC diagnosed in 76% and endometrial cancer in 23.5% of these individuals. Many individuals were diagnosed with two or more cancer types (e.g. CRC and endometrial cancer, endometrial cancer and ovarian cancer etc.) or one cancer type plus colorectal polyps. The majority of individuals were female (61.5%, N=432) and the median age at genetic testing was 59.2 years.

Fifteen distinct abnormal IHC patterns were reported. This included six IHC patterns categorized as typical, involving proteins from only one MMR heterodimer pair, and nine categorized as atypical, involving proteins from both pairs (Table 2). In total, 84.8% (N=596/703) of reported IHC patterns were typical, with the most common being a lack of MLH1/PMS2 expression. Among the 15.2% (N=107/703) atypical patterns, the most common involved disrupted expression of all four MMR proteins. Since atypical IHC patterns were rare, they were combined for subsequent analyses.

TABLE 1 Characteristics of the testing population.

	Patient characteristics (N=703)
Age at testing (years)	
Mean	59.4
Median	59.2
Range	15.3-91.5
Gender [n (%)]	
Male	269 (38.3)
Female	432 (61.5)
Not specified	2 (0.3)
Personal cancer history[a], n (%)	
Colorectal	534 (76.0)
Endometrial	165 (23.5)
Ovarian	7 (1.0)
Other	119 (16.9)
Colorectal polyps	96 (13.7)
Not specified[c]	1 (0.1)
Age at diagnosis (years)[b], n (%)	
≤40	81 (12.1)
41-50	136 (20.4)
51-60	163 (24.4)
61-70	142 (21.3)
>70	130 (19.5)
Not specified	15 (2.2)

[a]Individuals with multiple cancer diagnoses are included in each appropriate row.
[b]Earliest age at cancer diagnosis; includes only individuals with colorectal, endometrial, or ovarian cancer.
[c]Tumor type is not specified on the test request form.

10.3389/fonc.2023.1069467

TABLE 2 Immunohistochemistry patterns for MMR proteins among tested individuals.

	n (%)
Typical IHC patterns[a]	596 (84.8)
MLH1/PMS2	265 (37.7)
MSH2/MSH6	103 (14.7)
MSH6	92 (13.1)
PMS2	72 (10.2)
MLH1	40 (5.7)
MSH2	24 (3.4)
Atypical IHC patterns[b]	107 (15.2)
MLH1/MSH2/MSH6/PMS2	34 (4.8)
MLH1/MSH6/PMS2	17 (2.4)
MSH6/PMS2	23 (3.3)
MLH1/MSH2	8 (1.1)
MLH1/MSH2/MSH6	10 (1.4)
MSH2/MSH6/PMS2	7 (1.0)
MLH1/MSH2/PMS2	4 (0.6)
MLH1/MSH6	2 (0.3)
MSH2/PMS2	2 (0.3)
Total	**703**

[a]Typical patterns involved only one of the two MMR heterodimers (MSH2/MSH6 or MLH1/PMS2).
[b]Atypical patterns involved two or three MMR proteins that were from both MMR heterodimer pairs.
IHC, immunohistochemistry; MMR, mismatch repair.

Germline PV identification

Among all the individuals included in this study, 23.2% (N=163/703; 95% CI, 20.1%-26.5%) carried germline PVs in MMR genes (Table 3). Expected PVs in MMR genes were seen in 21.3% individuals (N=150/703). Among the 163 PV carriers, 8.0% (N=13/163; 95% CI, 4.3%-13.3%) carried PVs in unexpected MMR genes. No individual carried more than one PV, and no *EPCAM* PVs were identified in this cohort. Monoallelic PVs in *MUTYH* were identified in 6 individuals (Supplemental Table 2). These were excluded from the analysis because only biallelic *MUTYH* PVs are considered as high risk for CRC (29, 30).

Table 3 shows the distribution of IHC patterns among individuals found to have expected or unexpected MMR-gene PVs. It appears that PVs in expected MMR genes occurred most frequently in individuals showing isolated loss of MSH6 on IHC (45.7%; N=42/92) and least frequently among those showing isolated loss of MLH1 (7.5%; N=3/40). The most frequent unexpected MMR findings were observed in individuals who had isolated loss of MSH2 on IHC (20.8%; N=5/24). No unexpected MMR mutations were found in individuals with loss of MSH6 or PMS2 on IHC.

Table 4 lists the 13 individuals with unexpected germline PVs. The most common unexpected finding was germline PVs in *MSH6* in individuals with isolated loss of MSH2 on IHC, which was seen in 4 out of 5 individuals.

Germline VUS in MMR genes

199 MMR-gene VUSs were identified in nearly a quarter of the cohort (24.0%; N=169/703; 95% CI, 20.9%-27.4%) and some patients harbored more than one VUS. In 121 patients, 132 of these VUSs occurred in MMR genes that are expected to be mutated based on the IHC test result (17.2%; N=121/703; 95% CI,14.5%-20.2%).

For these 132 VUSs, there was a lack of sufficient evidence to support pathogenic or benign classifications at the time of variant identification (IHC was not considered as evidence for classification). During the timeline of this data query, May 2011 through April 2018, 44.7% (N=59/132; 95% CI, 36.0%-53.6%) of these observed VUSs were re-classified to likely benign or benign using evidence independent of IHC results, affecting 47.1% (N=57/121) individuals. By contrast, only 12.9% (N=17/132) of these VUSs were re-classified to PVs, affecting 14.0% (17/121) individuals (Table 5). The percentages of re-classified VUSs varied between different IHC patterns. For example, in individuals with isolated loss of PMS2 on IHC, 55.6% of *PMS2* VUSs were downgraded to benign/likely benign variants. However, only 16.7% *MLH1* VUSs were downgraded to benign/likely benign in patients with isolated loss of MLH1. On the other hand, only 3.6% of *MSH6* VUSs were upgraded to PVs in patients with loss of MSH6 in IHC; however, 50% of *MSH2* VUSs found in individuals with loss of MSH2 were determined to be pathogenic (Table 5).

These 132 VUSs represented 103 unique variants, 35 of which were downgraded and 14 of which were upgraded (Table 6). InSiGHT guidelines indicate that IHC can be considered as evidence in variant classification if a VUS is observed in at least two patients when the gene is expected to be mutated based on IHC results (23). To assess the potential impact of IHC results in variant classification consistent with InSiGHT criteria, we evaluated the variants in genes expected to be mutated based on IHC in at least two patients. Of the 103 unique VUS, 17 were observed in at least 2 patients. Among these 17 variants that were classified as VUSs at the time of identification, 70.6% (N=12/17; 95% CI, 44.0%-89.7%) were downgraded to benign/likely benign and 17.6% (N=3/17; 95% CI, 3.8%-43.4%) were upgraded to pathogenic/likely pathogenic. The downgrade affected 77.8% (N=35/45; 95% CI, 62.9%-88.8%) of patients, and the upgrade affected 13.3% (N=6/45; 95% CI, 5.1%-26.8%) of patients (Table 6).

The evidence used for downgrading a VUS to benign/likely benign included an in-house cancer history weighting algorithm (Pheno) (31), in-trans observation with a PV in patients with no features of constitutional mismatch repair deficiency (CMMRD) syndrome (phase), functional RNA studies (splicing), updated population frequency estimate (population), and in-house algorithm for using multiple co-occurrence for evidence of pathogenicity called MCO (28, 31). Table 7 lists the basis for downgrade for these 35 variants. The most frequently used evidence was Pheno, accounting for downgrade of 17 variants. Downgrading based on phase (i.e., in-trans findings) in patients without clinical features of CMMRD was used for 13 variants. Seven variants were downgraded using population frequency.

Discussion

In this study, we analyzed germline findings in 703 individuals with Lynch syndrome-associated cancer types and abnormal IHC findings to evaluate the concordance of IHC with the germline

TABLE 3 Expected or unexpected germline pathogenic variants (PVs) identified among individuals tested with the six-gene panel, distributed by IHC pattern and type of variant.

	Tested Individuals			
IHC pattern	Total	With PV N (%)	Individuals with PV in expected[a] MMR gene N (% of 703)	Individuals with PV in unexpected[a] MMR gene N (% of 703)
Typical				
MLH1/PMS2	265	31 (11.7)	28 (10.6%)	3 (1.1%)
MSH2/MSH6	103	33 (32.0)	31 (30.1%)	2 (1.9%)
MSH6	92	42 (45.7)	42 (45.7%)	0 (0%)
PMS2	72	23 (31.9)	23 (31.9%)	0 (0%)
MLH1	40	5 (12.5)	3 (7.5%)	2 (5%)
MSH2	24	10 (41.7)	5 (20.8%)	5 (20.8%)
Atypical[b]	107	19 (17.8)	18 (16.8%)	1 (0.9%)
Total	703	163 (23.2)	150 (21.3%)	13 (1.8%)

[a]Based on IHC result.
[b]Atypical IHC patterns are listed in Table 2.

TABLE 4 Unexpected germline mismatch repair gene pathogenic variants found in individuals with different immunohistochemistry patterns.

	Germline PV				
IHC pattern	*MLH1*	*MSH2*	*MSH6*	*PMS2*	Total
MLH1	0	2	0	0	2
MLH1/MSH2/PMS2	0	0	1	0	1
MLH1/PMS2	0	2	1	0	3
MSH2	0	0	4	1	5
MSH2/MSH6	1	0	0	1	2
Total	1	4	6	2	13

Monoallelic MUTYH PVs are provided in Supplemental Table 2.

TABLE 5 Germline MMR gene VUSs that were observed in at least one individual with consistent IHC according to IHC pattern.

	Observed variants			Individuals carrying the variants		
IHC pattern	Total	Downgraded to Benign/Likely Benign	Upgraded to Pathogenic/Likely Pathogenic	Total	Downgraded to Benign/Likely Benign	Upgraded to Pathogenic/Likely Pathogenic
Typical						
MLH1; PMS2	42	22 (52.4%)	5 (11.9%)	40	22 (55.0%)	5 (12.5%)
MSH2; MSH6	21	9 (42.9%)	4 (19.0%)	20	8 (40.0%)	4 (20.0%)
MSH6	28	8 (28.6%)	1 (3.6%)	24	8 (23.3%)	1 (4.2%)
PMS2	18	10 (55.6%)	4 (22.2%)	15	9 (60.0%)	4 (26.7%)
MLH1	6	1 (16.7%)	1 (16.7%)	5	1 (20.0%)	1 (20.0%)
MSH2	2	1 (50.0%)	1 (50.0%)	2	1 (50.0%)	1 (50.0%)
Atypical[a]	15	8 (44.4%)	1 (6.7%)	15	8 (44.4%)	1 (6.7%)
Total	132	59 (44.7%)	17 (12.9%)	121	57 (47.1%)	17 (14.0%)

[a]Atypical IHC patterns are listed in Table 2.

TABLE 6 Germline MMR gene VUS that were reclassified and patients affected by reclassification.

	Consistent IHC in ≥ 1 Patient	Consistent IHC in ≥ 2 Patient
Unique variants		
Total	103	17
Downgraded to Benign/Likely Benign, N (%)	35 (34.0%)	12 (70.6%)
Upgraded to Pathogenic/Likely Pathogenic, N (%)	14 (13.6%)	3 (17.6%)
Total patients with expected MMR variants (includes multiple observations of the same variant)		
Total	121	45
Downgraded to Benign/Likely Benign, N (%)	57 (47.1%)	35 (77.8%)
Upgraded to Pathogenic/Likely Pathogenic, N (%)	17 (14.0%)	6 (13.3%)

findings. To our knowledge, this is the first study to show the prevalence and spectrum of germline MMR gene mutations detected in a heterogeneous population with abnormal IHC that are referred to a commercial molecular diagnostic laboratory.

Within the entire cohort, only 21.3% of the individuals carried PVs in MMR genes predicted by IHC. This is lower than the previously reported germline PV positive rates in CRC patients with combined IHC and somatic BRAF testing (13). Somatic BRAF mutation p.V600E is present in 69% of methylation cases (32), which can contribute to the absence of MLH1/PMS2 on IHC. This study is solely based on the IHC results provided by the health care provider on the test requisition forms without any information on the somatic *BRAF* mutation status, therefore many of the cases with MLH1/PMS2 missing on IHC may be resulting from a somatic *BRAF* mutation. This might contribute to the lower PV positive rate in our cohort as we did not have information on somatic *BRAF* mutation status.

Overall, 8% of PV carriers identified by our panel testing carry a PV in an MMR gene not predicted by IHC results. This is particularly prevalent in individuals with isolated loss of MSH2 by IHC, where a single gene testing strategy would lead to *MSH2* sequencing. Of the 10 individuals with loss of MSH2 by IHC and PV positive in this cohort, 5 harbored PVs in genes other than *MSH2*. These findings suggests that IHC-guided single gene testing can extend the patient's diagnostic journey, potentially miss a Lynch syndrome diagnosis, and delay appropriate medical management. Therefore, MMR gene panel tests should be offered to all patients with abnormal IHC to prevent missing a Lynch syndrome diagnosis.

Our data showed that over 17% of patients with abnormal IHC had a VUS initially identified in the MMR gene predicted to be mutated by IHC. More importantly, nearly 1/3 of these variants were observed in more than one individual with concordant IHC results, which would be considered supporting evidence for a class 5 (pathogenic) or a class 4 (likely pathogenic) classification according to InSiGHT guidelines (23). However, in nearly half of these patients, the VUSs identified were downgraded to benign variants. If IHC results had been used as evidence for pathogenicity, these variants might have been classified in error as likely pathogenic, potentially leading to unnecessary overtreatment in the form of intensified screening and risk-reducing surgeries. These findings warrant great caution for the use of IHC results as evidence of pathogenicity for variants in MMR genes.

We observed 15 different IHC patterns in our patient cohort, 9 of which we considered "atypical" because proteins from both MLH1/PMS2 and MSH2/MSH6 heterodimers were affected. It has been reported that the IHC-null phenotype, in which all four MMR proteins are absent, can be caused by a combination of *MLH1* promoter methylation and double somatic mutation in *MSH2* (33). Double somatic mutations have been demonstrated as an important mechanism affecting MMR protein expression (13, 34, 35). We suspect that many of these atypical IHC patterns in our cohort are caused by this mechanism, affecting both heterodimers; or in certain cases by a combination of a germline mutation affecting one heterodimer and double somatic mutation affecting the other heterodimer. Some of these IHC patterns, such as isolated loss of MLH1, may represent certain artifacts due to antibody reactivity (36). Nevertheless, the findings of PVs in these cases underscores the necessity of testing all MMR genes for patients suspected for Lynch syndrome, irrespective of the IHC results.

One limitation of the analysis was the assumption that the IHC results reported on the test request form were accurate and that results were based on a staining method that included all four MMR proteins. In practice, to reduce costs, laboratories often begin with two-protein staining for MSH6 and PMS2, with reflex to MSH2 and/or MLH1 if a defect is detected. The rationale for two-protein staining stems from the fact that the stabilities of MSH6 and PMS2 depend largely on their dimerization with MSH2 and MLH1, respectively. However, it has been shown that the two-staining method can miss a small number of Lynch syndrome patients who have solitary loss of MSH2 (37). Based on this observation, patients with intact PMS2 or MSH6, but isolated loss of MLH1 or MSH2 may have been inadvertently excluded from this patient cohort. However, these findings are considered rare, and since they are not included in the concordance calculation, we do not anticipate these patients to greatly impact our conclusion. Another limitation of our study is the lack of information of the *MLH1* promoter methylation status. Promoter methylation assays often are conducted for individuals showing loss of MLH1 and/or PMS2 patterns, and only upon testing negative for promoter methylation would these individuals be referred for germline genetic testing (17, 38, 39). However, *MLH1* promoter methylation status was unknown for individuals in this study since

TABLE 7 35 variants downgraded from VUS to Benign/Likely benign.

Gene	Variant	Current classification	Evidence used for downgrade
MLH1	c.977T>C (p.Val326Ala)	PM	Pheno and MCO
MSH6	c.3203G>A (p.Arg1068Gln)	Likely benign	MCO
MLH1	c.1321G>A (p.Ala441Thr)	PM	MCO and Phase
MSH2	c.1465G>A (p.Glu489Lys)	PM	MCO and Pheno
MSH6	c.3173-18T>A	PM	MCO and Pheno
MSH6	c.-18G>T	PM	MCO and Phase
PMS2	c.2149G>A (p.Val717Met)	Likely benign	Phase
PMS2	c.2356C>A (p.Leu786Met)	Likely benign	Phase
MLH1	c.1732-19T>A	PM	Phase
MLH1	c.1897-17C>G	PM	Phase, Pheno
MSH2	c.815C>T (p.Ala272Val)	PM	Phase, Pheno
MSH2	c.1168C>T (p.Leu390Phe)	PM	Phase
MLH1	c.1360G>C (p.Gly454Arg)	PM	Pheno
MSH6	c.1474A>G (p.Met492Val)	Likely benign	Pheno
MSH2	c.877A>G (p.Thr293Ala)	Likely benign	Pheno
MLH1	c.2066A>G (p.Gln689Arg)	Likely benign	Pheno
MLH1	c.1268G>A (p.Arg423Lys)	Likely benign	Pheno
MSH6	c.1844G>C (p.Cys615Ser)	Likely benign	Pheno
MSH2	c.160G>T (p.Ala54Ser)	Likely benign	Pheno
MLH1	c.1667+4A>G	Likely benign	Pheno
MSH2	c.1600C>T (p.Arg534Cys)	Likely benign	Pheno
PMS2	c.251-20T>G	Likely benign	Population
PMS2	c.52A>G (p.Ile18Val)	PM	Population
MLH1	c.307-19A>G	PM	Splicing
MLH1	c.1963A>G (p.Ile655Val)	PM	MCO,Phase
MSH6	c.3160A>T (p.Ile1054Phe)	PM	Population
PMS2	c.1437C>G (p.His479Gln)	PM	Population
PMS2	c.1711C>A (p.Leu571Ile)	PM	Population
MSH2	c.380A>G (p.Asn127Ser)	PM	Phase,Pheno
MSH2	c.1321A>C (p.Thr441Pro)	PM	Phase,Pheno
MSH6	c.2633T>C (p.Val878Ala)	PM	Phase
PMS2	c.1609G>A (p.Glu537Lys)	PM	Population
MSH6	c.3488A>T (p.Glu1163Val)	PM	Population
MSH2	c.1387-8G>T	PM	Phase
MSH2	c.1277-8T>C	PM	Pheno

methylation assay information was not captured on the laboratory's test request form. Therefore, the low PV-positive rates correlating with loss of MLH1 or concurrent loss of MLH1/PMS2 on IHC might reflect *MLH1* promoter methylation in some individuals.

In conclusion, the overall germline PV positive rate of abnormal IHC in a population of patients with Lynch-associated cancer types who were referred to a clinical molecular diagnostic lab is approximately 20%, and nearly 2% of these individuals carried a germline PV in an MMR gene that was not consistent with the IHC result. In addition, VUS findings in genes that appear consistent with IHC findings were often downgraded to benign based on independent evidence. These findings raise two clinical issues. First, in currently

recommended testing procedures there exists true risk for missing germline PVs in Lynch syndrome, as well as other conditions that may predispose patients to hereditary cancers and have characteristics overlapping the hallmarks of Lynch syndrome. The outcome can be misdiagnosis and undertreatment of patients. Second, IHC results are not reliable as supportive evidence for variant classification. Our findings support revisiting guideline recommendations for diagnostic testing of individuals diagnosed with CRC or other Lynch syndrome-related cancers with consideration given to first-line use of comprehensive germline panel testing that combines analytical accuracy with robust variant classification. This recommendation is aligned with the recent update of the NCCN guideline to consider germline multigene panel testing for all individuals with CRC (3).

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Data are not publicly available due to patient privacy concerns, but can be provided upon reasonable request from the corresponding author. Requests to access these datasets should be directed to Shujuan Pan, span@myriad.com.

Ethics statement

Ethical review and approval was not required for the study of human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants OR patients/participants legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

SP, JW, EM, DM-D, and BC contributed to conceptualization. HC contributed to data curation. HC contributed to formal analysis. SP contributed to methodology and project administration. HG, ML, KB, BR and DMD contributed to supervision. KB contributed to validation. SP wrote the original draft, and JW, KB and DM-D contributed additional writing, review, and editing. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by Myriad Genetic Laboratories, Inc.

Acknowledgments

We would like to thank Sarah Ratzel, PhD, Elizabeth Cogan, PhD, Krystal Brown, PhD and Jennifer Logan, PhD for editing and reviewing the manuscript.

Conflict of interest

All authors were employees of Myriad Genetic Laboratories, Inc., at the time of this work and received salary and stock options as compensation.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1069467/full#supplementary-material

References

1. Kastrinos F, Samadder NJ, Burt RW. Use of family history and genetic testing to determine risk of colorectal cancer. *Gastroenterology* (2020) 158(2):389–403. doi: 10.1053/j.gastro.2019.11.029

2. Nguyen LH, Goel A, Chung DC. Pathways of colorectal carcinogenesis. *Gastroenterology* (2020) 158(2):291–302. doi: 10.1053/j.gastro.2019.08.059

3. Gupta S, Weiss JM, Axell L, et al. *NCCN clinical practice guidelines in oncology: Genetic/Familial high-RIsk assessment: Colorectal (Version 1.2022)* (2022). National Comprehensive Cancer Network. Available at: https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf (Accessed August 19, 2022).

4. Engel C, Rahner N, Schulmann K, Holinski-Feder E, Goecke TO, Schackert HK, et al. Efficacy of annual colonoscopic surveillance in individuals with hereditary nonpolyposis colorectal cancer. *Clin Gastroenterol hepatol: Off Clin Pract J Am Gastroenterol Assoc* (2010) 8(2):174–82. doi: 10.1016/j.cgh.2009.10.003

5. Järvinen HJ, Mecklin JP, Sistonen P. Screening reduces colorectal cancer rate in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology* (1995) 108 (5):1405–11. doi: 10.1016/0016-5085(95)90688-6

6. Järvinen HJ, Renkonen-Sinisalo L, Aktán-Collán K, Peltomäki P, Aaltonen LA, Mecklin JP. Ten years after mutation testing for lynch syndrome: Cancer incidence and outcome in mutation-positive and mutation-negative family members. *J Clin Oncol* (2009) 27(28):4793–7. doi: 10.1200/JCO.2009.23.7784

7. Sepulveda AR, Hamilton SR, Allegra CJ, Grody W, Cushman-Vokoun AM, Funkhouser WK, et al. Molecular biomarkers for the evaluation of colorectal cancer: Guideline from the American society for clinical pathology, college of American pathologists, association for molecular pathology, and the American society of clinical oncology. *J Clin Oncol* (2017) 35(13):1453–86. doi: 10.1200/JCO.2016.71.9807

8. Stoffel EM, Mangu PB, Gruber SB, Hamilton SR, Kalady MF, Lau MW, et al. 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. *J Clin Oncol* (2015) 33(2):209–17. doi: 10.1200/JCO.2014.58.1322

9. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Screening for the lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* (2005) 352(18):1851–60. doi: 10.1056/NEJMoa043146

10. Vasen HF, Moslein G, Alonso A, Bernstein I, Bertario L, Blanco I, et al. Guidelines for the clinical management of lynch syndrome (hereditary non-polyposis cancer). *J Med Genet* (2007) 44(6):353–62. doi: 10.1136/jmg.2007.048991

11. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* (2004) 96(4):261–8. doi: 10.1093/jnci/djh034

12. Parsons MT, Buchanan DD, Thompson B, Young JP, Spurdle AB. 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. *J Med Genet* (2012) 49(3):151–7. doi: 10.1136/jmedgenet-2011-100714

13. Hampel H, Pearlman R, Beightol M, Zhao W, Jones D, Frankel WL, 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(6):806–13. doi: 10.1001/jamaoncol.2018.0104

14. Noll A, JP P, Zhou M, Weber TK, Ahnen D, Wu XC, et al. Barriers to lynch syndrome testing and preoperative result availability in early-onset colorectal cancer: A national physician survey study. *Clin Transl Gastroenterol* (2018) 9(9):185. doi: 10.1038/s41424-018-0047-y

15. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. part i. the utility of immunohistochemistry. *J Mol Diagn.* (2008) 10(4):293–300. doi: 10.2353/jmoldx.2008.080031

16. Bartley AN, Luthra R, Saraiya DS, Urbauer DL, Broaddus RR. Identification of cancer patients with lynch syndrome: Clinically significant discordances and problems in tissue-based mismatch repair testing. *Cancer Prev Res (Phila).* (2012) 5(2):320–7. doi: 10.1158/1940-6207.CAPR-11-0288

17. Buchanan DD, Clendenning M, Rosty C, Eriksen SV, Walsh MD, Walters RJ, et al. Tumor testing to identify lynch syndrome in two Australian colorectal cancer cohorts. *J Gastroenterol Hepatol* (2017) 32(2):427–38. doi: 10.1111/jgh.13468

18. Roth RM, Haraldsdottir S, Hampel H, Arnold CA, Frankel WL. Discordant mismatch repair protein immunoreactivity in lynch syndrome-associated neoplasms: A recommendation for screening Synchronous/Metachronous neoplasms. *Am J Clin Pathol* (2016) 146(1):50–6. doi: 10.1093/ajcp/aqw067

19. Espenschied CR, LaDuca H, Li S, McFarland R, Gau CL, Hampel H. Multigene panel testing provides a new perspective on lynch syndrome. *J Clin Oncol* (2017) 35 (22):2568–75. doi: 10.1200/JCO.2016.71.9260

20. Yurgelun MB, Allen B, Kaldate RR, Bowles KR, Judkins T, Kaushik P, et al. Identification of a variety of mutations in cancer predisposition genes in patients with suspected lynch syndrome. *Gastroenterology* (2015) 149(3):604–13. doi: 10.1053/j.gastro.2015.05.006

21. Lorans M, Dow E, Macrae FA, Winship IM, Buchanan DD. Update on hereditary colorectal cancer: Improving the clinical utility of multigene panel testing. *Clin Colorectal Cancer.* (2018) 17(2):e293–305. doi: 10.1016/j.clcc.2018.01.001

22. Tricarico R, Kasela M, Mareni C, Thompson BA, Drouet A, Staderini L, et al. Assessment of the InSiGHT interpretation criteria for the clinical classification of 24 MLH1 and MSH2 gene variants. *Hum Mutat* (2017) 38(1):64–77. doi: 10.1002/humu.23117

23. Tumours ISfGH. the InSiGHT variant interpretation committee: Mismatch repair gene variant classification criteria. Available at: https://www.insight-group.org/content/uploads/2018/08/2018-06_InSiGHT_VIC_v2.4.pdf (Accessed 10/21/2020, 2020).

24. Ferguson SE, Aronson M, Pollett A, Eiriksson LR, Oza AM, Gallinger S, et al. Performance characteristics of screening strategies for lynch syndrome in unselected women with newly diagnosed endometrial cancer who have undergone universal germline mutation testing. *Cancer* (2014) 120(24):3932–9. doi: 10.1002/cncr.28933

25. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Feasibility of screening for lynch syndrome among patients with colorectal cancer. *J Clin Oncol* (2008) 26(35):5783–8. doi: 10.1200/JCO.2008.17.5950

26. Mancini-DiNardo D, Judkins T, Woolstenhulme N, Burton C, Schoenberger J, Ryder M, et al. Design and validation of an oligonucleotide microarray for the detection of genomic rearrangements associated with common hereditary cancer syndromes. *J Exp Clin Cancer Res* (2014) 33(1):74. doi: 10.1186/s13046-014-0074-9

27. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American college of medical genetics and genomics and the association for molecular pathology. *Genet med: Off J Am Coll Med Genet* (2015) 17(5):405–24. doi: 10.1038/gim.2015.30

28. Esterling L, Wijayatunge R, Brown K, Morris B, Hughes E, Pruss D, et al. Impact of a cancer gene variant reclassification program over a 20-year period. *JCO Precis Oncol* (2020) 4:944–54. doi: 10.1200/PO.20.00020

29. Win AK, Dowty JG, Cleary SP, Kim H, Buchanan DD, Young JP, et al. Risk of colorectal cancer for carriers of mutations in MUTYH, with and without a family history of cancer. *Gastroenterology* (2014) 146(5):1208–1211.e1201-1205. doi: 10.1053/j.gastro.2014.01.022

30. Lubbe SJ, Di Bernardo MC, Chandler IP, Houlston RS. Clinical implications of the colorectal cancer risk associated with MUTYH mutation. *J Clin Oncol* (2009) 27 (24):3975–80. doi: 10.1200/JCO.2008.21.6853

31. Morris B, Hughes E, Rosenthal E, Gutin A, Bowles KR. Classification of genetic variants in genes associated with lynch syndrome using a clinical history weighting algorithm. *BMC Genet* (2016) 17(1):99. doi: 10.1186/s12863-016-0407-0

32. Palomaki GE, McClain MR, Melillo S, Hampel HL, Thibodeau SN. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from lynch syndrome. *Genet med: Off J Am Coll Med Genet* (2009) 11 (1):42–65. doi: 10.1097/GIM.0b013e31818fa2db

33. Wang T, Stadler ZK, Zhang L, Weiser MR, Basturk O, Hechtman JF, et al. Immunohistochemical null-phenotype for mismatch repair proteins in colonic carcinoma associated with concurrent MLH1 hypermethylation and MSH2 somatic mutations. *Fam Cancer.* (2018) 17(2):225–8. doi: 10.1007/s10689-017-0031-9

34. Mensenkamp AR, Vogelaar IP, van Zelst-Stams WA, Goossens M, Ouchene H, Hendriks-Cornelissen SJ, et al. Somatic mutations in MLH1 and MSH2 are a frequent cause of mismatch-repair deficiency in lynch syndrome-like tumors. *Gastroenterology* (2014) 146(3):643–646.e648. doi: 10.1053/j.gastro.2013.12.002

35. Sourrouille I, Coulet F, Lefevre JH, Colas C, Eyries M, Svrcek M, et al. Somatic mosaicism and double somatic hits can lead to MSI colorectal tumors. *Fam Cancer.* (2013) 12(1):27–33. doi: 10.1007/s10689-012-9568-9

36. Bosch DE, Yeh MM, Salipante SJ, Jacobson A, Cohen SA, Konnick EQ, et al. Isolated MLH1 loss by immunohistochemistry because of benign germline MLH1 polymorphisms. *JCO Precis Oncol* (2022) 6:e2200227. doi: 10.1200/PO.22.00227

37. Pearlman R, Markow M, Knight D, Pritchard CC, Hampel H, Frankel WL, et al. Two-stain immunohistochemical screening for lynch syndrome in colorectal cancer may fail to detect mismatch repair deficiency. *Mod Pathol* (2018) 31(12):1891–900. doi: 10.1038/s41379-018-0058-y

38. Buchanan DD, Tan YY, Walsh MD, Clendednning M, Metcalf AM, Ferguson K, et al. Tumor mismatch repair immunohistochemistry and DNA MLH1 methylation testing of patients with endometrial cancer diagnosed at age younger than 60 years optimizes triage for population-level germline mismatch repair gene mutation testing. *J Clin Oncol* (2014) 32(2):90–100. doi: 10.1200/JCO.2013.51.2129

39. Goodfellow PJ, Billingsley CC, Lankes HA, Ali S, Cohn DE, Broaddus RJ, et al. Combined microsatellite instability, MLH1 methylation analysis, and immunohistochemistry for lynch syndrome screening in endometrial cancers from GOG210: An NRG oncology and gynecologic oncology group study. *J Clin Oncol* (2015) 33(36):4301–8. doi: 10.1200/JCO.2015.63.9518

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 01 February 2023
DOI 10.3389/fonc.2023.1123901

Check for updates

OPEN ACCESS

EDITED BY
Jianxun J. Song,
Texas A&M Health Science Center,
United States

REVIEWED BY
Jiaqiao Fan,
Dalian Medical University, China
Anil Kumar,
Texas A&M Health Science Center,
United States

*CORRESPONDENCE
Kristina Zalevskaja
kristina.zalevskaja@helsinki.fi
Toni T. Seppälä
toni.seppala@helsinki.fi

SPECIALTY SECTION
This article was submitted to
Cancer Genetics,
a section of the journal
Frontiers in Oncology

RECEIVED 14 December 2022
ACCEPTED 18 January 2023
PUBLISHED 01 February 2023

CITATION
Zalevskaja K, Mecklin J-P and Seppälä TT (2023) Clinical characteristics of pancreatic and biliary tract cancers in Lynch syndrome: A retrospective analysis from the Finnish National Lynch Syndrome Research Registry.
Front. Oncol. 13:1123901.
doi: 10.3389/fonc.2023.1123901

COPYRIGHT
© 2023 Zalevskaja, Mecklin and Seppälä. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Clinical characteristics of pancreatic and biliary tract cancers in Lynch syndrome: A retrospective analysis from the Finnish National Lynch Syndrome Research Registry

Kristina Zalevskaja[1,2]*, Jukka-Pekka Mecklin[3,4] and Toni T. Seppälä[1,5]*

[1]Applied Tumor Genomics Research Program, Research Programs Unit, University of Helsinki, Helsinki, Finland, [2]Department of Gastrointestinal Surgery, Helsinki University Central Hospital, Helsinki, Finland, [3]Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland, [4]Department of Education and Research, Jyväskylä Hospital Nova, Jyväskylä, Finland, [5]Faculty of Medicine and Health Technology and Tays Cancer Centre, University of Tampere, Tampere, Finland

Introduction: Patients with Lynch syndrome (LS) have an increased lifetime risk of pancreatic cancer (PC) and biliary tract cancer (BTC). These cancers have a notoriously pessimistic prognosis due to late diagnosis and limited therapeutic options. There are limited data based on small cohorts reviewing PC and BTC in LS patients.

Methods: In this retrospective study of the Lynch Syndrome Registry of Finland (LSRFi), records of genetically verified LS patients diagnosed with PC or BTC between 1982 and 2020 were analyzed.

Results: Thirty-nine patients were included: tumor(s) were in the pancreas in 26 patients, in the biliary tract in 10, and in the ampulla of Vater in three. A pathogenic germline variant was found in *MLH1* in 33 of 39 patients. Twenty-six patients with 28 tumors located in the pancreas were identified: 23 pancreatic ductal adenocarcinomas (PDACs) and five neuroendocrine tumors (NETs). The median age at diagnosis of PC was 64 years (range of 38–81). In PC, the 5-year overall survival (OS) rate was 20%, and in PDAC, it was 13.6%. Ten patients with BTC were diagnosed: two intrahepatic, five perihilar, two distal extrahepatic cholangiocarcinomas, and one gallbladder carcinoma. Eight patients were male, and the median age at diagnosis was 54 years (range of 34–82). The 5-year OS rate for BTC was 30%. Metachronous tumors were diagnosed in 28 patients (70%). Colorectal cancer was the most common metachronous tumor, diagnosed in 20 patients (51%), and diagnosed prior to PC or BTC in all cases. Curative surgery was attempted on 17 of 39 patients. For 30 patients (91%), the cause of death was PC or BTC; two patients died from another LS-associated cancer, and one died from a stroke.

Conclusion: Although the survival of LS patients with PC or BTC is better than in sporadic cancers, it is still poor and may be reflected by the relatively higher

surgical resectability accounted for by the earlier age of onset. More studies on analyses of the molecular and immune profile, screening, and management of LS-associated pancreaticobiliary cancers are warranted.

KEYWORDS

Lynch syndrome (LS), hereditary nonpolyposis colon cancer (HNPCC), pancreatic cancer, biliary tract cancer, microsatellite instability (MSI)

1 Introduction

Lynch syndrome (LS), previously known as hereditary nonpolyposis colorectal cancer (HNPCC), is an autosomal dominant disorder caused by pathogenic germline variants in one of the DNA mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6*, or *PMS2*, or by deletions in the *EPCAM* gene (1–3). It is the most common hereditary cancer syndrome, with a prevalence estimated as high as 1 in 279 (4). Pathogenic MMR variant carriers have a high lifetime risk of developing colorectal and endometrial cancers and an increased risk of developing gastric, ovarian, urothelial, pancreatic, biliary tract, small bowel, prostate, breast, brain, and skin cancers, depending on the gene affected (5). LS-associated cancers usually display MMR deficiency (dMMR) that leads to microsatellite instability (MSI) in the tumors.

Increased risk of pancreatic cancer (PC) in LS carriers was first observed by Lynch et al. in 1985 (6). In 1992, Mecklin et al. described 11 LS patients with biliary tract cancer (BTC), suggesting an association between BTC and LS (7). Since then, numerous retrospective studies and one review have confirmed an increased incidence of PCs and BTCs in LS patients (8–12). The Prospective Lynch Syndrome Database (PLSD) report has shown different lifetime risks for PC depending on the germline mutation variant (5).

LS-associated colorectal, gynecological, and gastric cancers have a better prognosis than sporadic cancers (5, 13–16). Unfortunately, PC and BTC remain aggressive and have a poor prognosis. As of lately, immune checkpoint inhibitor therapy has been an exciting development in the treatment of solid tumors with MSI and dMMR, including PC and BTC, with promising results (17–20). However, there are limited data based only on small cohorts reviewing pancreatic and biliary tract malignancies in LS patients.

In this article, we present the largest cohort of LS patients with PC and BTC to date and characterize their clinical features.

2 Methods

2.1 Study cohort

We retrospectively reviewed the medical records of patients in the Lynch Syndrome Registry of Finland (LSRFi) who were diagnosed with pancreatic or biliary tract malignant tumors between 1982 and 2020. The nationwide registry, established in 1982, includes, at present, 1,800 verified pathogenic variant carriers from 400 families and contains clinicopathological information on all cancers of registered individuals. The data have been regularly cross-checked against the Finnish national cancer registry.

This multicenter retrospective study was approved by the national authority for registry research (Findata), waiving the requirement for informed consent to use data obtained from medical records.

2.2 Survival analyses

Overall survival (OS) was defined as the time from diagnosis until death from any cause or the last date of confirmed survival. OS was analyzed in R using the Kaplan–Meier method (21).

2.3 Pathological classification

Pancreatic adenocarcinomas (PDACs) were graded according to histopathological WHO criteria (22). Pancreatic neuroendocrine tumors (NETs) were classified according to the WHO 2020 classification for pancreatic neuroendocrine neoplasms (23). Biliary tract tumors were classified according to system based on their anatomical location and categorized as intrahepatic, perihilar, or distal cholangiocarcinomas (24). According to the WHO classification of digestive system tumors, adenocarcinomas of the ampulla of Vater are histologically closer to the small intestine but anatomically near the pancreas and biliary tract (22). Therefore, this rare type of cancer was included in the cohort but analyzed separately.

3 Results

3.1 Patient characteristics

Forty LS patients were diagnosed with PC or BTC or ampullary cancer between 1982 and 2020 (Table 1). Among them, tumors in 26 patients were in the pancreas, 10 in the biliary tract, and three in the ampulla of Vater. One patient was excluded from the study due to non-pancreatic and non-biliary histology. A pathogenic germline variant of *MLH1* was detected in 33 patients, *MSH2* in five, and *MSH6* in one patient. *MLH1* variant carriers had 21 out of 26 PCs, nine out of 10 BTC, and all three ampullary cancers (Figure 1).

LS was diagnosed prior to PC or BTC in 22 patients and simultaneously in 14 patients. Each patient's family in LSRFi and the number of LS-diagnosed patients in this family are presented in

TABLE 1 Clinicopathological characteristics of PC, BTC, and ampullary cancer in LS patients.

Cancer type	Age	Sex	Family id	LS patients in the family	Primary site	Histology	Operation	Chemotherapy	Radiation therapy	Metachronous tumors	Gene	OS (month)
Ampulla of Vater	65	F	4	4	Ampulla of Vater	Ampullary adenocarcinoma	R0	no	no	CRC×3, uterus, ovary, ventricle	*MLH1*	27
Ampulla of Vater	53	F	1	31	Ampulla of Vater	Ampullary adenocarcinoma	R0	yes	yes		*MLH1*	32
Ampulla of Vater	49	M	87	10	Ampulla of Vater	Ampullary adenocarcinoma	R0	NA	NA	CRC×2, prostate	*MLH1*	275
Biliary tract	48	F	73	24	Gallbladder	Carcinoma of gallbladder	R0	no	no		*MLH1*	Alive
Biliary tract	53	M	19	7	Common bile duct	Cholangiocarcinoma	palliative	no	yes	CRC	*MLH1*	6
Biliary tract	80	F	231	7	Common bile duct	Cholangiocarcinoma	R0	NA	NA	Uterus, ureter	*MSH6*	Alive
Biliary tract	50	M	195	6	Intrahepatic	Cholangiocarcinoma	no	yes	no		*MLH1*	9
Biliary tract	68	M	205	4	Intrahepatic	Cholangiocarcinoma	R0	no	no	CRC×4	*MLH1*	Alive
Biliary tract	55	M	1	31	Perihilar	Cholangiocarcinoma	no	no	no	CRC	*MLH1*	31
Biliary tract	82	M	160	4	Perihilar	Cholangiocarcinoma	no	no	no	CRC×2	*MLH1*	15
Biliary tract	40	M	99	7	Perihilar	Cholangiocarcinoma	no	yes	no	CRC	*MLH1*	9
Biliary tract	34	M	50	34	Perihilar	Cholangiocarcinoma	R1	yes	no		*MLH1*	27
Biliary tract	74	M	61	21	Perihilar	Cholangiocarcinoma	no	yes	yes	CRC	*MLH1*	11
Pancreas	47	M	94	3	head	Ductal adenocarcinoma	no	yes	no		*MLH1*	12
Pancreas	81	F	78	7	head	Ductal adenocarcinoma	no	yes	no		*MLH1*	9
Pancreas	58	M	2	37	head	Ductal adenocarcinoma	palliative	no	no	CRC×2	*MLH1*	1
Pancreas	69	M	9	6	head	Ductal adenocarcinoma	R0	no	no	CRC, ureter	*MLH1*	1
Pancreas	54	F	157	2	head	Ductal adenocarcinoma	no	yes	yes	CRC, uterus	*MLH1*	6
Pancreas	67	F	2	37	head	Ductal adenocarcinoma	R1	yes	no	Uterus, ovary	*MLH1*	Alive
Pancreas	61	M	152	3	head	Ductal adenocarcinoma	R0	no	no	Spinal cord	*MLH1*	129
Pancreas	64	F	54	29	head	Ductal adenocarcinoma	no	no	no	CRC×2, ovary	*MLH1*	3
Pancreas	69	M	38	12	head	Ductal adenocarcinoma	no	yes	no		MSH2	7
Pancreas	68	M	1	31	head	Ductal adenocarcinoma	R0	no	no	CRC, prostate	*MLH1*	8
Pancreas	74	F	112	21	head	Ductal adenocarcinoma	no	no	no	CRC×2, uterus	*MLH1*	6

(Continued)

10.3389/fonc.2023.1123901

TABLE 1 Continued

Cancer type	Age	Sex	Family id	LS patients in the family	Primary site	Histology	Operation	Chemotherapy	Radiation therapy	Metachronous tumors	Gene	OS (month)
Pancreas	70	F	24	2	head	Ductal adenocarcinoma	palliative	no	no	Ureter, acusticus neurinoma	*MLH1*	2
Pancreas	86	F	241	4	body	Ductal adenocarcinoma	no	no	no	CRC, uterus	*MLH1*	0
Pancreas	60	M	82	12	body	Ductal adenocarcinoma	R1	yes	no		*MLH1*	32
Pancreas	45	F	98	11	body	Ductal adenocarcinoma	no	yes	no	CRC	*MLH1*	36
Pancreas	80	F	105	10	body	Ductal adenocarcinoma	palliative	yes	no	CRC×2, uterus	*MLH1*	4
Pancreas	53	F	122	3	body	Ductal adenocarcinoma	no	yes	no	Cervix	MSH2	7
Pancreas	71	F	138	2	body	Ductal adenocarcinoma	R0	no	no	Uterus	*MLH1*	Alive
Pancreas	54	F	191	2	body	Ductal adenocarcinoma, NET	R1	yes	no	Breast	MSH2	6
Pancreas	39	F	23	6	tail	Ductal adenocarcinoma	R1	yes	no	Ovary, ventricle	*MLH1*	5
Pancreas	66	M	19	7	tail	Ductal adenocarcinoma	no	no	no	CRC	*MLH1*	1
Pancreas	56	M	10	11	tail	Ductal adenocarcinoma	palliative	no	yes	CRCx2	*MLH1*	15
Pancreas	64	M	146	4	NA	Ductal adenocarcinoma	no	no	no		*MLH1*	3
Pancreas	63	F	1	31	body	NET	no	yes	no		*MLH1*	2
Pancreas	38	F	191	2	body, tail	NETx2	R0	no	no	CRC	*MSH2*	101
Pancreas	69	M	38	12	tail	NET	R1	yes	no		*MSH2*	Alive

NA, not available; CRC, colorectal cancer; NET, neuroendocrine tumor; R0, negative resection margin; R1, positive resection margin; OS, overall survival.

10.3389/fonc.2023.1123901

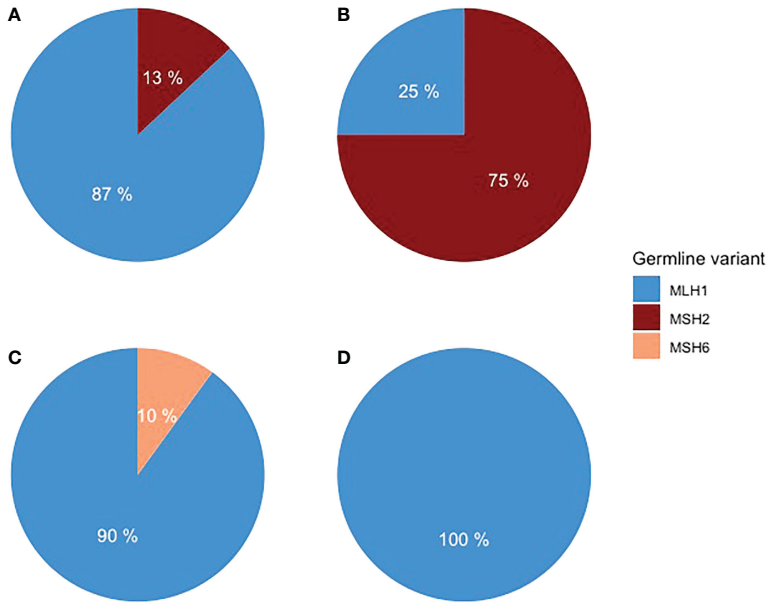


FIGURE 1
Distribution of germline variants in **(A)** PDAC, **(B)** pancreatic NET, **(C)** BTC, and **(D)** ampullary adenocarcinoma.

Table 1. Five families had more than one family member diagnosed with PC or BTC.

In 26 patients with 28 tumors located in the pancreas, 23 were PDAC and five were NETs. Fifteen were female, and the median age at diagnosis was 64 years (range of 38–81). The distribution of anatomical locations of PC was head 12, body 10, and tail five (one location was not recorded). Germline variants of *MLH1* were detected in 21 patients and *MSH2* in five patients. One patient had simultaneously PDAC and NET, and one patient had two NETs. In both patients, multiple endocrine neoplasia type 1 (MEN1) syndrome was additionally diagnosed. The diagnosis of pancreatic malignancies in 20 patients was based on clinical, radiological, and pathology reports, but in six patients, it was based on clinical and radiological findings only.

Of the 10 BTC patients, two were intrahepatic, five perihilar, two distal extrahepatic cholangiocarcinomas, and one gallbladder carcinoma. Eight were male, and the median age at diagnosis was 54 years (ranging from 34 to 82). Germline variants of *MLH1* were detected in nine patients and of *MSH6* in one patient. The BTC diagnosis was based on a pathology report in seven patients and on computer tomography in three patients. The patient with gallbladder carcinoma was primarily operated on due to pain caused by gallstones but received an unexpected diagnosis of gallbladder carcinoma. The treatment was later completed with liver segment II–IV resections with R0 margins.

Adenocarcinoma of the ampulla of Vater was diagnosed in three patients, all *MLH1* carriers. Two were women, and the median age at diagnosis was 53 years (ranging from 49 to 65). As all three patients underwent surgery, the diagnosis was verified by a pathological report.

3.2 Metachronous tumors

Metachronous tumors were diagnosed in 28 (72%) patients (median number 1; range of 0–6). Colorectal cancer was the most common, diagnosed in 20 patients, and endometrial cancer in eight patients. In all cases, colorectal cancer and endometrial cancer were diagnosed prior to PC or BTC.

3.3 Treatment and survival

Curative surgery was attempted in 17 (43.5%) patients and treatment with non-curative intent, life-prolonging or palliative therapy, was provided to 22 patients (Figure 2).

Ten out of 26 patients with PC underwent surgery with curative intent. Five patients had surgical resection with a negative resection margin. Five patients' resection margins were positive, and adjuvant chemotherapy was administered to these patients. In LS patients with PC who were treated with curative intent, the 5-year survival rate was

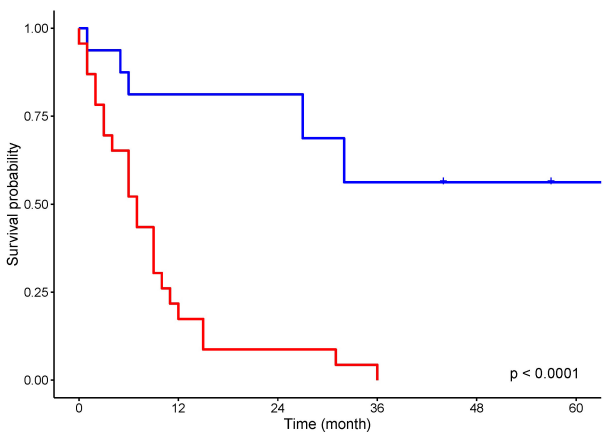


FIGURE 2
Kaplan–Meier analysis for overall survival of patients treated with curative intent (blue) and non curative intent (red).

50%. In the 10 LS patients with PDAC resected with curative intent, the 5-year survival rate was 38%. Seven patients were treated with chemotherapy and one patient with chemoradiation therapy. One patient received additional immunotherapy with pembrolizumab for MLH1/PMS2-deficient PDAC. Eight patients were provided with symptomatic treatment. In 16 patients who were not treated with curative intent, the median OS was 5 months. For all patients with PC, the 5-year OS rate was 20%, and for those with PDAC, it was 13.6% (Figure 3A). One patient died from pneumonia after a palliative operation within 72 h and was excluded from survival calculations. Endoscopic stent placement was performed in eight cases and percutaneous transhepatic drainage in one.

Four patients with BTC underwent surgical resection with curative intent. Three patients' resection margin was negative. One patient's resection margin was positive, and adjuvant chemotherapy was administered. Among these four patients with BTC who were operated on with curative intent, the 5-year survival rate was 75%. Two patients were treated with chemotherapy, and one with chemoradiation therapy. Three patients were treated symptomatically. In six patients who were not treated with curative intent, the median OS was 10 months. For LS patients with BTC, the 5-year OS rate was 30% (Figure 3B). Endoscopic stent placement was performed in three cases, and percutaneous transhepatic drainage was placed in four cases.

All three patients with adenocarcinomas of the ampulla of Vater underwent curative surgery with a negative resection margin. Still two patients died of recurrence of ampullary cancer within 1.5 years and one from small bowel cancer.

Thirty-three patients (85%) out of 39 were deceased. For thirty patients (91%), the cause of death was PC, BTC, or ampullary cancer. Two patients died from another LS-associated cancer and one patient died from a stroke. An overview of clinical characteristics and treatment is presented in Table 2.

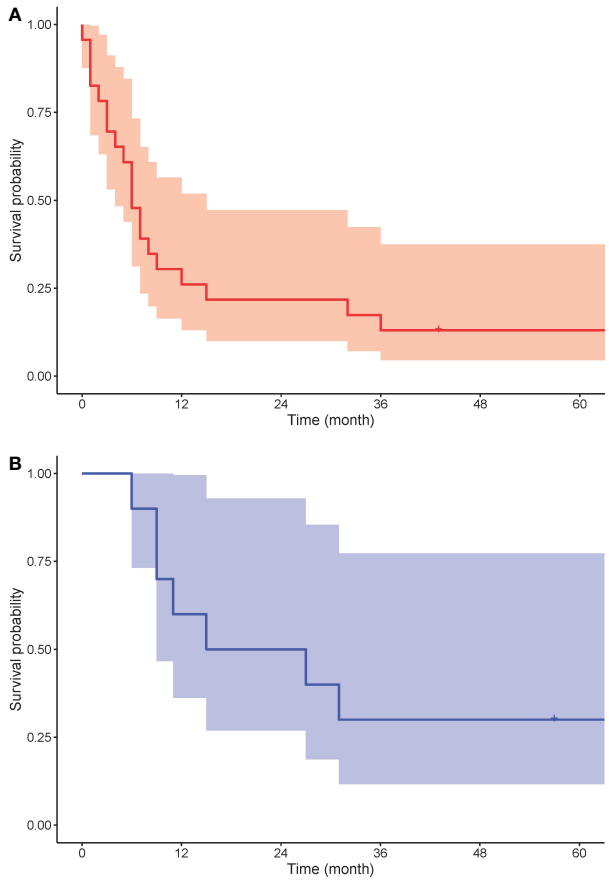


FIGURE 3
Kaplan-Meier survival analysis for overall survival of **(A)** PDAC (red) and **(B)** BTC (blue).

4 Discussion

Sporadic PCs and BTCs have a dismal prognosis due to being asymptomatic in the early stages, resulting in a late diagnosis. Treatment options are limited and lack effectiveness. The five-year survival rate is only 10% for both cancer types in the United States (25, 26). LS patients have an increased lifetime risk of developing PC and BTC. Analysis of data from the Prospective Lynch Syndrome Database (PLSD) has shown the cumulative risk at 75 years of age for PC is 6.2%, 0.5%, and 1.4%, and for BTC is 3.7%, 1.7%, and 0%, respectively, for carriers of *MLH1*, *MSH2*, and *MSH6* germline variants (5). Our retrospective study supports pathogenic *MLH1* germline variant carriers being overrepresented among LS patients with PC and BTC.

We did not detect any pancreaticobiliary cancers in *PMS2* carriers, although the number of identified *PMS2* families is low in Finland. No clear evidence of an increased risk of PC or BTC has been shown in *PMS2* pathogenic variant carriers, even in the larger series (27). In a study by Møller et al., none of the 124 *PMS2* pathogenic variant carriers were diagnosed with PC or BTC (5). Hu et al. reported three *PMS2* carriers with PDAC. Two of these patients developed MMR-proficient PDAC (28). Ando et al. performed immunohistochemistry (IHC) analysis on 116 operated BTC patients, identifying two *PMS2* germline variant carriers, both microsatellite stable (MSS) (29). These findings suggest that PDAC and BTC in *PMS2* germline mutation carriers might be sporadic. The cautionary tale of Wang et al. raises the importance of routine tumor testing for both MMR deficiency and MSI to detect patients who might have a better chance of responding to immunotherapy (30). Hendifar et al. presented a case report underscoring the importance of testing every cancer in LS patients for MMR, as not all of them might respond to immunotherapy (31).

The incidence of sporadic PC and BTC increases with age and the median age at the diagnosis is 70 years (32). In the current study, LS patients with PC or BTC were younger, resembling the early age phenomenon which is typical for all LS associated cancers. Also, PC was diagnosed equally in females and males. The small sample size of this study does not allow definitive conclusions drawn, but the latest PLSD report did report substantial sex difference in upper gastrointestinal cancers with 22% in male *MLH1* carriers by 75 years versus 11% in female *MLH1* carriers (33).

Three-quarters of LS patients with PC or BTC had metachronous tumors. Colorectal and endometrial cancers were diagnosed in all cases prior to PC or BTC. These findings suggest that most, but not all, LS patients develop PC or BTC later in life after the more common primary cancers. A personal cancer history of LS carriers over 60 years of age may serve as an indicator for healthcare to stay alert for unspecific symptoms the upper gastrointestinal cancers may induce. On the other hand, a quarter of the patients did not have a previous cancer history, and PC or BTC was their first malignancy.

TABLE 2 Overview of clinical characteristics and treatment in LS patients with pancreatic, biliary tract and ampullary cancer.

	Pancreatic cancer N=26		Biliary tract cancer N=10		Ampullary cancer N=3	
Age median, years *(range)*	64 *(39-81)*		54 *(34-80)*		53 *(49-65)*	
Sex						
M	11 *(42%)*		8 *(80%)*		1 *(33%)*	
F	15 *(58%)*		2 *(20%)*		2 *(67%)*	
Genes						
MLH1	21		9		3	
MSH2	5		0		0	
MSH6	0		1		0	
PMS2	0		0		0	
Histology						
	Ductal adenocarcinoma	23 *(82%)*	Cholangiocarcinoma	9 *(90%)*	Adenocarcinoma	3 *(100%)*
	NET	5 *(18%)*	Carcinoma of gallbladder	1 *(10%)*		
Primary site						
	Head	12	Intrahepatic	2	Ampulla of Vater	3
	Body	10	Perihilar	5		
	Tail	5	Common bile duct	2		
	Not Available	1	Gallbladder	1		
Metachronous tumors						
Number median *(range)*		1 *(0-3)*		1 *(0-4)*		3 *(0-6)*
Colorectal cancer		17		10		5
Endometrial cancer		6		1		1
Treatment						
Curative intent		10 *(38%)*		4 *(40%)*		3 *(100%)*
Non curative intent		16 *(62%)*		6 *(60%)*		0
5-year survival	20%		30%		33%	

Most sporadic PCs are in the head of the pancreas. PC in the body or tail has a worse prognosis compared to pancreatic head cancer due to remaining asymptomatic for a longer period, resulting in late diagnoses (34, 35). Takamizawa et al. described the anatomical location of the PC in six LS patients, identifying five of the six PCs as being in the body and the tail of the pancreas (36). In our study, PC was located equally often in the head, the body, and the tail of the pancreas. Our series suggests that no distinct primary anatomical site is more prevalent. BTC was found in all parts of the biliary tract, as also previously shown by Cloyd et al. (11).

Surgical resection is the only curative treatment for PC and BTC. Curative surgery can be performed in 10%–20% of sporadic PC cases and in 20% of sporadic BTC cases (37, 38). In this study, curative surgical resection was performed twice as often, resulting in better overall survival outcomes. This might be explained by the fact that half of the cases already had an LS diagnosis and participated in regular surveillance. In Finland, surveillance for PC and BTC in LS patients is symptom-based, as European guidelines for LS recommend (13). In practice, it means LS patients are educated about symptoms they might encounter and are encouraged to contact secondary and tertiary healthcare providers with expertise in LS if they experience any "red flag" symptoms.

Immune modulation therapy with checkpoint inhibition is a new promising option for LS-associated cancer types with poor prognosis, as histology-agnostic FDA approval for any dMMR or MSI solid cancers is in place (18). Pancreaticobiliary cancers are often deemed unresectable, but a proper molecular pathological examination revealing MSI with even some response to checkpoint inhibition may convert an inoperable case back to operable. However, good biopsies for histology might be difficult to get, and especially known LS carriers should be referred to experienced centers with high volumes of endoscopic ultrasound-guided biopsies to avoid false-negative biopsies for dMMR and MSI due to poor sample quality. It is especially important to not suffice with imaging or cytology-informed diagnosis alone, but a diagnostic biopsy for dMMR or MSI testing must be obtained in all cases with known or suspected LS.

This study has several limitations, such as a retrospective design, a small sample size, and, in some cases, a lack of pathological verification of cancer. Even though the small sample size of this cohort limits the power of statistical analysis, it is still the largest series reported to date. Although it seems that the survival of LS patients with PC or BTC is better than in sporadic cancers, it is still poor. The relatively higher surgical resectability may be accounted for by selection bias due to the earlier age of onset.

To conclude, there is a growing need for molecular and immune profiling of LS-associated PDAC and BTC to clarify the suitability of these cancers with an extremely poor prognosis for immune or any other upcoming therapy.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

KZ: Data curation, formal analysis, writing—original draft. J-PM: Conceptualization, supervision, writing—review and editing. TS: Supervision, writing—review and editing. All authors contributed to the article and approved the submitted version.

Funding

The research of the group was funded by the Jane and Aatos Erkko Foundation, Cancer Foundation Finland, Sigrid Juselius Foundation, Emil Aaltonen Foundation, Relander Foundation, Academy of Finland, iCAN Digital Precision Medicine flagship, and the State research funding.

Acknowledgments

The authors would like to acknowledge Maija Röntynen and Kirsi Pylvänäinen for help with the Lynch Syndrome Registry of Finland (LSRFi). We would like to thank Erdogan Pekcan Erkan for proofreading the article.

Conflict of interest

TS reports consultation fees from Boehringer Ingelheim Finland and Amgen Finland and being a co-owner and CEO of Healthfund Finland.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Lynch H, Lynch P, Lanspa S, Snyder C, Lynch J, Boland C. Review of the lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. *Clin Genet* (2009) 76(1):1–18. doi: 10.1111/j.1399-0004.2009.01230.x

2. Ligtenberg MJL, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, et al. Heritable somatic methylation and inactivation of MSH2 in families with lynch syndrome due to deletion of the 3′ exons of TACSTD1. *Nat Genet* (2009) 41(1):112–7. doi: 10.1038/ng.283

3. Kovacs ME, Papp J, Szentirmay Z, Otto S, Olah E. Deletions removing the last exon of TACSTD1 constitute a distinct class of mutations predisposing to lynch syndrome. *Hum Mutation.* (2009) 30(2):197–203. doi: 10.1002/humu.20942

4. Win AK, Jenkins MA, Dowty JG, Antoniou AC, Lee A, Giles GG, et al. Prevalence and penetrance of major genes and polygenes for colorectal cancer. *Cancer Epidemiol Biomarkers Prev* (2017) 26(3):404–12. doi: 10.1158/1055-9965.EPI-16-0693

5. Møller P, Seppälä TT, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the prospective lynch syndrome database. *Gut* (2018) 67(7):1306–16. doi: 10.1136/gutjnl-2017-314057

6. Lynch HT, Voorhees GJ, Lanspa SJ, McGreevy PS, Lynch JF. Pancreatic carcinoma and hereditary nonpolyposis colorectal cancer: a family study(1985). Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1977116/ (Accessed April 6, 2022).

7. Mecklin JP, Järvinen HJ, Virolainen M. The association between cholangiocarcinoma and hereditary nonpolyposis colorectal carcinoma. *Cancer* (1992) 69(5):1112–4. doi: 10.1002/cncr.2820690508

8. Kastrinos F, Stoffel EM, Balmaña J, Steyerberg EW, Mercado R, Syngal S. Phenotype comparison of MLH1 and MSH2 mutation carriers in a cohort of 1,914 individuals undergoing clinical genetic testing in the united states. *Cancer Epidemiology Biomarkers Prev* (2008) 17(8):2044–51. doi: 10.1158/1055-9965.EPI-08-0301

9. Geary J, Sasieni P, Houlston R, Izatt L, Eeles R, Payne SJ, et al. Gene-related cancer spectrum in families with hereditary non-polyposis colorectal cancer (HNPCC). *Familial Cancer.* (2008) 7(2):163–72. doi: 10.1007/s10689-007-9164-6

10. Borelli I, Casalis Cavalchini GC, Del Peschio S, Micheletti M, Venesio T, Sarottoet I, et al. A founder MLH1 mutation in lynch syndrome families from piedmont, Italy, is associated with an increased risk of pancreatic tumours and diverse immunohistochemical patterns. *Fam Cancer.* (2014) 13(3):401–13. doi: 10.1007/s10689-014-9726-3

11. Cloyd JM, Chun YS, Ikoma N, Vauthey JN, Aloia TA, Cuddy A, et al. Clinical and genetic implications of DNA mismatch repair deficiency in biliary tract cancers associated with lynch syndrome. *J Gastrointest Cancer.* (2018) 49(1):93–6. doi: 10.1007/s12029-017-0040-9

12. Bujanda L, Herreros-Villanueva M. Pancreatic cancer in lynch syndrome patients. *J Cancer.* (2017) 8(18):3667–74. doi: 10.7150/jca.20750

13. Seppälä TT, Latchford A, Negoi I, Sampaio Soares A, Jimenez-Rodriguez R, Sanchez-Guillén L, et al. European Guidelines from the EHTG and ESCP for lynch syndrome: an updated third edition of the mallorca guidelines based on gene and gender. *Br J Surgery.* (2021) 108(5):484–98. doi: 10.1002/bjs.11902

14. Crosbie EJ, Ryan NAJ, Arends MJ, Bosse T, Burn J, Corneset JM, et al. The Manchester international consensus group recommendations for the management of gynecological cancers in lynch syndrome. *Genet Med* (2019) 21(10):2390–400. doi: 10.1038/s41436-019-0489-y

15. Ryan N a. J, Evans DG, Green K, Crosbie EJ. Pathological features and clinical behavior of lynch syndrome-associated ovarian cancer. *Gynecologic Oncol* (2017) 144 (3):491. doi: 10.1016/j.ygyno.2017.01.005

16. Møller P, Seppälä T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Cancer incidence and survival in lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective lynch syndrome database. *Gut* (2017) 66(3):464–72. doi: 10.1136/gutjnl-2015-309675

17. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch-repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* (2017) 357 (6349):409–13. doi: 10.1126/science.aan6733

18. Marcus L, Lemery SJ, Keegan P, Pazdur R. FDA Approval summary: Pembrolizumab for the treatment of microsatellite instability-high solid tumors. *Clin Cancer Res* (2019) 25(13):3753–8. doi: 10.1158/1078-0432.CCR-18-4070

19. Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz H-J, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair deficient/microsatellite instability–high colorectal cancer (CheckMate 142): results of an open-label, multicentre, phase 2 study. *Lancet Oncol* (2017) 18(9):1182–91. doi: 10.1016/S1470-2045(17)30422-9

20. Zeng FL, Chen JF. Application of immune checkpoint inhibitors in the treatment of cholangiocarcinoma. *Technol Cancer Res Treat* (2021) 20:15330338211039952. doi: 10.1177/15330338211039952

21. R: The r project for statistical computing . Available at: https://www.r-project.org/ (Accessed May 5, 2022).

22. *Publication of WHO classification of tumours, 5th edition, volume 1: Digestive system tumours* . IARC. Available at: https://www.iarc.who.int/news-events/publication-of-who-classification-of-tumours-5th-edition-volume-1-digestive-system-tumours/ (Accessed May 5, 2022).

23. Khanna L, Prasad SR, Sunnapwar A, Kondapaneni S, Dasyam A, Tammisetti VS, et al. Pancreatic neuroendocrine neoplasms: 2020 update on pathologic and imaging findings and classification. *RadioGraphics* (2020) 40(5):1240–62. doi: 10.1148/rg.2020200025

24. Kendall T, Verheij J, Gaudio E, Evert M, Guido M, Goeppert B, et al. Anatomical, histomorphological and molecular classification of cholangiocarcinoma. *Liver Int* (2019) 39(S1):7–18. doi: 10.1111/liv.14093

25. *Pancreatic cancer - statistics* (2012). Cancer.Net. Available at: https://www.cancer.net/cancer-types/pancreatic-cancer/statistics (Accessed October 20, 2022).

26. *Bile duct cancer (Cholangiocarcinoma) - statistics* (2012). Cancer.Net. Available at: https://www.cancer.net/cancer-types/bile-duct-cancer-cholangiocarcinoma/statistics (Accessed October 20, 2022).

27. ten Broeke SW, van der Klift HM, Tops CMJ, Aretz S, Bernstein I, Buchanan DD, et al. Cancer risks for PMS2-associated lynch syndrome. *JCO* (2018) 36(29):2961–8. doi: 10.1200/JCO.2018.78.4777

28. Hu ZI, Shia J, Stadler ZK, Varghese AM, Capanu M, Salo-Mullen E, et al. Evaluating mismatch repair deficiency in pancreatic adenocarcinoma: Challenges and recommendations. *Clin Cancer Res* (2018) 24(6):1326–36. doi: 10.1158/1078-0432.CCR-17-3099

29. Ando Y, Kumamoto K, Matsukawa H, RYOU Ishikawa R, Suto H, Oshima M, et al. Low prevalence of biliary tract cancer with defective mismatch repair genes in a Japanese hospital-based population. *Oncol Lett* (2022) 23(1):4. doi: 10.3892/ol.2021.13122

30. Wang Y, Cuggia A, Pacis A, Boileau J-C, Marcuset VA, Gao ZH, et al. Pancreatic cancer progression in a patient with lynch syndrome receiving immunotherapy: A cautionary tale. *J Natl Compr Cancer Network*. (2021) 19(8):883–7. doi: 10.6004/jnccn.2021.7049

31. Hendifar AE, Larson BK, Rojansky R, Guan M, Gong J, Veronica Placencio V, et al. Pancreatic cancer 'mismatch' in lynch syndrome. *BMJ Open Gastroenterol* (2019) 6(1): e000274. doi: 10.1136/bmjgast-2019-000274

32. Wang H, Liu J, Xia G, Lei S, Huang X, Huang X. Survival of pancreatic cancer patients is negatively correlated with age at diagnosis: a population-based retrospective study. *Sci Rep* (2020) 10(1):7048. doi: 10.1038/s41598-020-64068-3

33. Dominguez-Valentin M, Sampson JR, Seppälä TT, ten Broeke SW, Plazzer J-P, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the prospective lynch syndrome database. *Genet Med* (2020) 22(1):15–25. doi: 10.1038/s41436-019-0596-9

34. Artinyan A, Soriano PA, Prendergast C, Low T, Ellenhorn JDI, Kim J. The anatomic location of pancreatic cancer is a prognostic factor for survival. *HPB (Oxford).* (2008) 10(5):371–6. doi: 10.1080/13651820802291233

35. Lee M, Kwon W, Kim H, Byun Y, Han Y, Kanget JS, et al. The role of location of tumor in the prognosis of the pancreatic cancer. *Cancers (Basel).* (2020) 12(8):2036. doi: 10.3390/cancers12082036

36. Takamizawa S, Morizane C, Tanabe N, Maruki Y, Kondo S, Susumu Hijioka S, et al. Clinical characteristics of pancreatic and biliary tract cancers associated with lynch syndrome. *J Hepatobiliary Pancreat Sci* (2022) 29(3):377–84. doi: 10.1002/jhbp.1063

37. Cillo U, Fondevila C, Donadon M, Gringeri E, Mocchegiani F, Schlitt HJ, et al. Surgery for cholangiocarcinoma. *Liver Int* (2019) 39(Suppl 1):143–55. doi: 10.1111/liv.14089

38. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet* (2011) 378(9791):607–20. doi: 10.1016/S0140-6736(10)62307-0

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 20 February 2023
DOI 10.3389/fonc.2023.1127707

Check for updates

OPEN ACCESS

EDITED BY
Toni T. Seppälä,
Helsinki University Central Hospital, Finland

REVIEWED BY
Priyanka Kanth,
MedStar Georgetown University Hospital, United States
Hisanaga Horie,
Jichi Medical University, Japan

*CORRESPONDENCE
Nigin Jamizadeh
nigin.jamizadeh@stud.ki.se

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 19 December 2022
ACCEPTED 25 January 2023
PUBLISHED 20 February 2023

CITATION
Jamizadeh N, Walton Bernstedt S, Haxhijaj A, Andreasson A, Björk J, Forsberg A and Backman A-S (2023) Endoscopic surveillance of Lynch syndrome at a highly specialized center in Sweden: An observational study of interval colorectal cancer and individual risk factors.
Front. Oncol. 13:1127707.
doi: 10.3389/fonc.2023.1127707

COPYRIGHT
© 2023 Jamizadeh, Walton Bernstedt, Haxhijaj, Andreasson, Björk, Forsberg and Backman. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Endoscopic surveillance of Lynch syndrome at a highly specialized center in Sweden: An observational study of interval colorectal cancer and individual risk factors

Nigin Jamizadeh[1*†], Sophie Walton Bernstedt[1,2,3†], Adrianna Haxhijaj[1], Anna Andreasson[1], Jan Björk[2,4], Anna Forsberg[5] and Ann-Sofie Backman[1,6]

[1]Unit of Gastroenterology, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden, [2]Division of Gastroenterology, Medical Unit Gastroenterology, Dermatovenereology and Rheumatology, Karolinska University Hospital, Stockholm, Sweden, [3]Division of Upper Gastrointestinal Diseases, Karolinska University Hospital, Stockholm, Sweden, [4]Hereditary Cancer Unit, Theme Cancer, Karolinska University Hospital, Stockholm, Sweden, [5]Division of Clinical Epidemiology, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden, [6]Division of Gastroenterology, Department of Medicine, Ersta Hospital, Stockholm, Sweden

Introduction: Lynch syndrome (LS) is the most common hereditary cause of colorectal cancer (CRC). In order to detect CRCs amongst LS patients, regular colonoscopies are recommended. However, an international agreement on an optimal surveillance interval has not yet been reached. In addition, few studies have investigated factors that could potentially increase the CRC risk amongst LS patients.

Aims: The primary aim was to describe the frequency of CRCs detected during endoscopic surveillance and to estimate the interval from a clean colonoscopy to CRC detection amongst LS patients. The secondary aim was to investigate individual risk factors, including sex, LS genotype, smoking, aspirin use and body mass index (BMI), on CRC risk amongst patients that develop CRC before and during surveillance.

Material and methods: Clinical data and colonoscopy findings from 366 LS patients' 1437 surveillance colonoscopies were collected from medical records and patient protocols. Logistic regression and Fisher's exact test were used to investigate associations between individual risk factors and CRC development. Mann-Whitney U test was used to compare the distribution of TNM stages of CRC detected before surveillance and after index.

Results: CRC was detected in 80 patients before surveillance and in 28 patients during surveillance (10 at index and 18 after index). During the surveillance programme, CRC was detected within 24 months in 65% of the patients, and after 24 months within 35% of the patients. CRC was more common amongst men, previous and current smokers, and the odds of developing CRC also increased with

an increasing BMI. CRCs were more often detected amongst *MLH1* and *MSH2* carriers during surveillance, compared to the other genotypes.

Conclusions: We found that 35% of the CRC cases detected during surveillance were found after 24 months. *MLH1* and *MSH2* carriers were at higher risk of developing CRC during surveillance. Additionally, men, current or previous smokers, and patients with a higher BMI were at higher risk of developing CRC. Currently, LS patients are recommended a "one-size-fits-all" surveillance program. The results support the development of a risk-score whereby individual risk factors should be taken into consideration when deciding on an optimal surveillance interval.

KEYWORDS

Lynch syndrome (LS), colorectal cancer, interval cancer, surveillance, colonoscopy

1 Introduction

Colorectal cancer (CRC) is the third most common cancer type diagnosed worldwide, after breast cancer and lung cancer. Causing approximately 1 million deaths annually, CRC is the second leading cause of cancer-related deaths in the world, following lung cancer (1). Genetic factors have a great impact on the risk of CRC development; up to 30% of CRCs have been estimated to have a familial component (2). Accounting for 3% of all CRCs, Lynch syndrome (LS) is the most common genetic cause of colorectal cancer. The risk of developing extracolonic cancers is higher amongst LS patients than non-LS patients (3). The most common extracolonic cancers amongst LS patients include endometrial cancer, gastric cancer, ovarian cancer, small bowel cancer, pancreas cancer, and cancer in the urothelial tract (4). LS is caused by germline mutations in the mismatch repair (MMR) genes *MLH1, MSH2, MSH6, PMS2*, and *EPCAM*, which are inherited in an autosomal dominant fashion (5).

Although it has been established that several behavioral and environmental factors are important risk modifiers for the development of CRC in the general population (6, 7), few studies have investigated the impact of lifestyle and individual factors on CRC risk amongst LS patients.

The association between smoking and CRC risk in LS is still somewhat uncertain. Pande et al. (8) conducted a retrospective cohort study of 752 LS patients and found no difference in CRC risk between ever- and never-smokers. When the ever-smokers were divided into current and previous smokers, an increased CRC risk was shown amongst current smokers, whereas previous smokers showed a decreased risk of CRC. Watson et al. (9) included smoking data from 360 LS patients in their retrospective study and found that tobacco users had a higher CRC risk than non-users. However, a limitation of their study was the lack of subcategories amongst tobacco users, as current smokers were not distinguished from previous smokers. In terms of BMI, several studies suggest that male sex combined with higher BMI leads to an increased CRC risk, whereas this association could not be found amongst females with higher BMI and LS (10–12).

Moreover, LS patients with different genotypes seem to have different risks of CRC development. For instance, some studies suggest that MLH1 carriers are at a higher risk of developing CRC than PMS2 carriers (13–15). Meanwhile, MLH1 and MSH2 carriers are estimated to have a similar risk for CRC, and MSH6 carriers present an intermediate risk in the genetic spectrum (16).

In order to detect CRCs amongst LS patients, regular colonoscopies are recommended. However, no international agreement on an optimal surveillance interval has yet been reached. Annual surveillance is recommended for MLH1 and MSH2 carriers in Australia (6) and every one to two years in the United States, starting from the age of 20–25 (5). Differences in surveillance intervals can also be observed in Europe, where the European Society of Gastrointestinal Endoscopy (ESGE) recommends biennial surveillance of asymptomatic LS patients (17). However, the recommended surveillance interval is every two to three years in Finland, every one to two years in the Netherlands, and annually in Germany (6). In Sweden, the recommended surveillance interval is every one to two years (18), and patients with LS in Stockholm undergo annual surveillance.

The life-saving effect of surveillance colonoscopies has been established by several studies. De Jong et al. (19) studied the surveillance interval program introduced in the Netherlands during the late 1980s. The mortality rate of CRC amongst LS patients was investigated before and after 1990, and the results showed a decrease in CRC mortality after 1990, supporting the efficiency of a one- to two-year surveillance program.

In a study published in 2019, Engel et al. (6) investigated the optimal surveillance interval amongst a total of 2747 MLH1, MSH2, and MSH6 carriers. Data were collected from 16,327 colonoscopies performed between 1984 and 2015. The study, which included LS patients from the Netherlands, Germany, and Finland, concluded that there was no significant difference in cumulative CRC incidence between the countries, although the recommended surveillance intervals varied from one to every three years.

Debate around the optimal interval for endoscopic surveillance of LS patients is ongoing: some studies suggest that shorter intervals are

more beneficial (19), whilst others suggest no difference in CRC risk amongst LS patients in countries with different surveillance intervals (6). It is therefore of great importance to study the endoscopic surveillance intervals of LS patients further, whilst analyzing individual factors affecting their CRC incidence risk, to identify risk patients who may benefit from shorter surveillance intervals.

The primary aim of this study was to study the frequency of CRCs detected during endoscopic surveillance and to estimate the interval from a clean colonoscopy to CRC detection amongst LS patients. The secondary aim was to investigate individual risk factors, including sex, LS genotype, smoking, aspirin use and BMI, on CRC risk amongst patients who develop CRC before and during surveillance.

2 Materials and methods

2.1 Study design and subjects

A single-center, observational cohort study was conducted at Karolinska University Hospital in Stockholm, Sweden. The study considered LS patients with an MMR gene mutation who were followed at the Karolinska University Hospital from 1989 to April 2021. All MMR gene mutations were confirmed according to the InSight Variant Committee's classification (20) or reported to be pathogenic by the hospital's genetics department if the variant was unknown. Of 427 LS patients registered at the clinic, 366 were eligible for inclusion. Part of the cohort has previously been described (18).

After the MMR gene mutation had been confirmed, patients were recommended an index colonoscopy within 3 months. If the colonoscopy was "clean", surveillance continued with a recommended interval of 1-2 years.

CRCs were classified as detected before surveillance if they had been detected before the MMR gene mutation was confirmed. A CRC detected at index or after index (where index is defined as the first colonoscopy after LS diagnosis is given, and after index is defined as the second colonoscopy after the MMR gene mutation is confirmed) was classified as detected during surveillance. Patients with multiple CRCs, detected both before and during surveillance, are also included in the "during surveillance" group below. Colonoscopies were counted and analyzed up until CRC detection. To study interval cancers only, the colonoscopies detecting CRC had to follow a "clean" examination within a reasonable timeframe. Accordingly, index CRCs were excluded from the TNM distribution and surveillance interval calculations. Underweight, normal weight, overweight and obese were defined as BMI (<18.5 kg/m^2), (18.5–24.9 kg/m^2), (18.5-<25.0 kg/m^2) and (25.0-<30.0 kg/m^2), respectively.

2.2 Data collection

Data were collected from medical records retrospectively and structured patient protocols prospectively during patient consultations. Questions about sex, age, height, weight, smoking habits, aspirin use and previous cancer(s) were answered in the protocol at the first gastroenterology outpatient visit after the LS diagnosis was ascertained. Data from the medical records included colonoscopy results from the patients' surveillance colonoscopies performed from 1989 to 2021. Additional data regarding the date of LS and CRC diagnosis, cause of LS and CRC diagnosis, LS genotype, TNM stage and localization of CRC, and the surgical procedure performed were collected from the medical records from 1975 to 2021.

2.3 Statistical analysis

Descriptive statistics are presented as numbers (n) and percentages (%) for categorical variables and as mean values and standard deviations (SD) for continuous variables. Univariable and multivariable logistic regression was used to investigate the associations between individual factors, including sex, BMI, smoking, aspirin use, LS genotype, and CRC development. Univariable logistic regression was used to test differences in sex, BMI, age at LS and CRC diagnosis, and smoking status between patients with CRC detected before and during surveillance, Fisher's exact test was used to test differences in LS genotypes between the two groups. The Mann–Whitney U test was used to compare the distribution of TNM stages of CRC detected before and during surveillance. Statistical significance was set at $p \leq 0.05$. All statistical calculations were performed in SPSS package 28 (IBM® SPSS Statistics® version 28) made for macOS.

2.4 Ethical considerations

Data were obtained from medical records and structured protocols filled in by the patients at the hospital. All the procedures being performed were part of the routine clinical care. Ethical approval was granted by the Regional Ethics Review Board in Stockholm, Sweden, with approval number 2017/2013-31/2 and the Swedish Ethical Review Authority with approval number 2022-00119-0.

3 Results

Of a total number of 1887 colonoscopies, 76% were performed within 24 months. Of these, 1437 were surveillance colonoscopies, registered up until the latest CRC detection.

There were 366 patients in the cohort, of which 108 had at least one CRC diagnosis (referred to as "CRC cohort" below). Of these, 80 had their CRC detected before the MMR gene mutation had been confirmed. Twenty-eight patients had their CRC detected during surveillance, of which ten had a CRC detected at index, eighteen after index and four had a CRC detected both before and during surveillance.

The patient characteristics of the study population in total, for those who developed CRC, and for those who had not developed CRC are presented in Table 1. In the total cohort, the mean age for LS diagnosis was 42 years, and the most common LS genotype was MLH1 (45%), followed by MSH2 (28%). More than half the total cohort were never-smokers (57%).

The logistic regression models investigating the association between potential risk factors and CRC development are presented

10.3389/fonc.2023.1127707

TABLE 1 Patient characteristics in total and separated by CRC status.

Variable	Total cohort (n=366) n (%)	CRC cohort (n=108) n (%)	Non-CRC cohort (n=258) n (%)
Sex			
Men	169 (46)	59 (55)	110 (43)
Women	197 (54)	49 (45)	148 (57)
Deceased	15 (4)	8 (7)	7 (3)
Age at death (mean ± SD)	63.9 ± 15.2	67.8 ± 14.8	59.4 ± 15.5
Genotype			
MLH1	164 (45)	55 (51)	109 (42)
MSH2	103 (28)	33 (30)	70 (27)
MSH6	51 (14)	12 (11)	39 (15)
PMS2	38 (10)	6 (6)	32 (12)
EPCAM	6 (2)	1 (1)	5 (2)
Mixed genotype	4 (1)	1 (1)	3 (1)
Age at diagnosis (mean ± SD)	42.0 ± 15.4	49.2 ± 13.2	39.1 ± 15.2
Smoking status			
Current smoker	36 (10)	15 (14)	21 (8)
Previous smoker	104 (28)	42 (39)	62 (24)
Never-smoker	210 (57)	50 (46)	160 (62)
Missing data	16 (4)	1 (1)	15 (6)
Use of aspirin			
Current or previous	47 (13)	27 (25)	20 (8)
Never	295 (81)	77 (71)	218 (84)
Missing data	24 (7)	4 (4)	20 (8)
BMI (mean ± SD)	25.4 ± 4.6	26.3 ± 5.1	25.0 ± 4.4
Age at CRC diagnosis (mean ± SD)		45.5 ± 12.6	

Data are presented as numbers and percentages for nominal variables and as mean values and standard deviations for continuous variables. BMI, Body Mass Index; CRC, Colorectal Cancer; SD, Standard Deviation.

in Table 2. In the univariable logistic regression, men had 62% higher odds of developing CRC than women, and patients with a PMS2 gene mutation had lower odds of developing CRC than MLH1 gene carriers (OR=0.37, 95% CI: 0.15–0.94). Smokers, both current and previous, had more than double the odds of patients who had never smoked. The odds of developing CRC also increased with an increasing BMI (OR=1.06, 95% CI: 1.01–1.12), whereas no significant difference could be found when comparing the BMI categories of underweight, overweight, and obese with normal BMI. Patients with current or previous aspirin use showed a significantly increased risk for CRC development than those who had never used it (OR=2.73, 95% CI: 1.51-4.94). The results from the multivariable logistic regression analysis demonstrate the same pattern, with the odds of developing CRC being significantly higher in men than women (OR=1.76, 95% CI: 1.08–2.87) as well as amongst current (OR=2.70, 95% CI: 1.21–6.03 and previous (OR=2.12, 95% CI: 1.26–3.59) smokers than never-smokers. Similarly, PMS2 carriers had lower odds of developing CRC than MLH1 carriers (OR=0.31, 95% CI: 0.12–0.81), and the results for BMI were identical in both logistic regression analyses. Additionally, patients with aspirin use had higher odds of CRC development than never-users.

Patient characteristics of the CRC cohort in total and separated for cancer detection before surveillance (n=80) and during surveillance (n=28) are presented in Table 3A. Univariable logistic regression analysis was used to investigate the associations between individual factors and CRC detection during surveillance as an outcome. There was no statistically significant difference in sex distribution between the “before surveillance” group and the “during surveillance” group. Due to the low numbers in some of the genotypes, MLH1 and MSH2 were compared to the MSH6, PMS2, EPCAM, and mixed genotype between CRCs detected before and during surveillance. Only MLH1 and MSH2 carriers developed CRCs during surveillance, whereas MSH6, PMS2, EPCAM carriers and patients with a mixed genotype did not develop CRC during surveillance (p=0.01). The age at LS diagnosis was significantly lower amongst patients with CRC detection during surveillance than before surveillance (OR=0.95, 95% CI: 0.91–0.98), while no statistical difference was found in the age at CRC diagnosis between

TABLE 2 Risk factors for developing CRC.

Variable	Crude OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*
Sex				
Men	1.62 (1.03-2.55)	0.04*	1.76 (1.08-2.87)	0.02*
Women	Ref	Ref	Ref	Ref
Genotype				
MLH1	Ref	Ref	Ref	Ref
MSH2	0.93 (0.55-1.58)	0.93	0.79 (0.45-1.38)	0.41
MSH6	0.61 (0.30-1.26)	0.18	0.51 (0.24-1.09)	0.08
PMS2	0.37 (0.15-0.94)	0.04*	0.31 (0.12-0.81)	0.02*
EPCAM	0.40 (0.05-3.48)	0.40	0.32 (0.03-3.19)	0.34
Mixed genotype	0.66 (0.07-6.50)	0.72	0.46 (0.05-4.64)	0.51
Smoking status				
Never-smoker	Ref	Ref	Ref	Ref
Previous smoker	2.17 (1.31-3.60)	<0.001*	2.12 (1.26-3.59)	0.05*
Current smoker	2.30 (1.10-4.77)	0.03*	2.70 (1.21-6.03)	0.02*
Use of aspirin				
Never	Ref	Ref	Ref	Ref
Current or previous	2.73 (1.51-4.94)	<0.001*	2.66 (1.41-5.01)	0.002*
BMI (linear)	1.06 (1.01-1.12)	0.01*	1.06 (1.01-1.12)	0.03*

BMI, Body Mass Index; CI, Confidence Interval; OR, Odds Ratio; Ref, Reference Variable. *Statistically significant values are marked with an asterisk.
Univariable (crude OR) and multivariable (adjusted OR) logistic regression.

patients with a CRC detection before surveillance (44.9 ± 12.3) and during surveillance (47.5 ± 13.6).

Of twenty-eight CRC cases detected during surveillance, ten CRC cases were detected at index and eighteen CRC cases were detected after index. The patient characteristics of the CRC cohort in total and separated for cancer detection before surveillance (n=80) and after index (n=18) are presented in Table 3B. There was a significant difference in LS genotype between the two groups, where only MLH1 and MSH2 carriers had CRC detection after index (p<0.001). The age at LS diagnosis was significantly lower amongst patients with CRC detection after index than before surveillance (OR=0.95, 95% CI:0.91–0.98).

TNM stages of the CRCs detected before surveillance and during surveillance are presented in Table 4. Of 108 CRC cases, data on TNM classification were available for 86 CRCs. Of 18 interval CRCs, 17 had a TNM classification. Before surveillance, 46% of the CRCs were classified as stage I and II, and after index, 67% of the CRCs were classified as stage I and II. No significant difference was found between the TNM stage distribution before surveillance compared to after index (p=0.5).

Surveillance intervals of the TNM-classified CRC cases found during surveillance are presented in Figure 1. Of the 17 CRC cases, 11 (65%) were detected within a 24-month interval. Two CRC cases were detected within 6 months, of which one had a shorter interval due to a suspect polyp with LGD, which turned out to be an adenocarcinoma. The second CRC case was detected due to post-surgical CRC symptoms, which turned out to be a relapse 6 months after the latest clean colonoscopy.

4 Discussion

In this study, we aimed to describe the frequency of CRCs detected during surveillance colonoscopies and estimate the interval from a clean colonoscopy to CRC detection amongst LS patients. We also aimed to evaluate individual risk factors, including sex, LS genotype, smoking, aspirin use and BMI, on CRC risk amongst patients who develop CRC before and during surveillance.

The results show that 80 of 108 CRC cases were detected before surveillance, and twenty-eight CRC cases were detected during surveillance. Of these, ten were index CRCs and eighteen were interval CRCs. Most of the CRC cases detected during surveillance were detected within a 24-month interval. We found that the risk of CRC detection during surveillance is significantly higher amongst MLH1 and MSH2 carriers than among MSH6, PMS2, and EPCAM carriers and patients with a mixed genotype. When studying individual risk factors, we found that current and previous smokers had almost twice as high odds of developing CRC as non-smokers, and patients who were male and had a higher BMI had higher odds of CRC development than women and patients with a lower BMI.

The ESGE's surveillance guidelines for asymptomatic LS patients recommends a surveillance interval of 24 months (17). Our results show

TABLE 3A Univariable logistic regression and Fisher's exact test on patient characteristics of CRC cohort before and during surveillance.

Variable	Before surveillance (n=80) n (%)	During surveillance (n=28) n (%)	Crude OR (95% CI)	*p*
Sex				
Female	36 (73)	13 (27)	Ref	Ref
Male	44 (75)	15 (25)	1.1 (0.45-2.51)	0.90
Deceased	5 (63)	3 (37)		
Age at death (mean ± SD)	66.6 ± 17.0	69.7 ± 13.3		
Genotype**				
MLH1	32 (58)	23 (42)		0.01*
MSH2	28 (85)	5 (15)		0.01*
MSH6	12 (100)	0 (0)		Ref
PMS2	6 (100)	0 (0)		Ref
EPCAM	1 (100)	0 (0)		Ref
Mixed genotype	1 (100)	0 (0)		Ref
Age at diagnosis (mean ± SD)	51.4 ± 13.1	42.9 ± 11.5	0.95 (0.91–0.98)	0.05*
Smoking status				
Current smoker	9 (60)	6 (40)	1.90 (0.57–6.37)	0.30
Previous smoker	33 (79)	9 (21)	0.78 (0.29–2.05)	0.61
Never-smoker	37 (74)	13 (26)	Ref	Ref
Missing data	1 (100)	0 (0)		
BMI (mean ± SD)	26.1 ± 4.5	27.0 ± 6.7	1.03 (0.95-1.12)	0.42
Age at CRC diagnosis (mean ± SD)	44.9 ± 12.3	47.5 ± 13.6	1.02 (0.98-1.05)	0.35

Data are presented as numbers and percentages for nominal variables and as mean values and standard deviations for continuous variables. BMI, Body Mass Index; CI, Confidence Interval; OR, Odds Ratio; Ref, Reference Variable; SD, Standard Deviation.
*Statistically significant values are marked with an asterisk.
** Genotypes in cursive and mixed genotype using Fisher's exact test.

that 65% of the CRC cases detected after index were detected within this interval (Figure 1). Around 35% of the CRC cases were detected within 24–37 months or longer, which could be explained by poor patient compliance or the organization's failure to adhere to hospital routines, leading to the postponement of colonoscopy appointments.

Contrary to both our initial expectations and a previous study by Engel et al. (21), we could not find a significant difference in TNM stage distribution between CRC cases detected before surveillance and after index. One possible explanation for this could be the small number of TNM-classified CRCs in the medical records of the LS patients. We did not detect a difference in TMN classification amongst CRCs detected within different colonoscopy intervals either. However, this result should be interpreted with caution as colonoscopy findings are subjective; therefore, a missed lesion at a "clean colonoscopy" could lead to a CRC with a higher TNM classification after a shorter interval.

In terms of risk factors, we found that men have a higher risk of developing CRC than women (Table 2). We also found that both previous and current smokers have higher odds of developing CRC than never-smokers, which is in line with the results Pande et al. presented in their study (9). In addition, our results show that an increase per BMI unit is associated with higher odds of CRC development, whereas no significant difference could be found within BMI categories of underweight, overweight, and obese when compared to normal BMI. This could partly be explained by the small sample size in the different BMI categories of the cohort. Previous studies have concluded that male sex combined with high BMI are risk factors for developing CRC, whereas this association could not be found amongst females with higher BMI and LS (11–13). We did not compare men and women when performing the logistic regression analysis on BMI. However, this could be of interest for future research.

Several randomized control trials have investigated the role of aspirin on adenoma recurrence, most of which have found a significant decrease in adenoma recurrence and CRC risk amongst aspirin-users (22–24). Interestingly, we found that patients with current or previous use of aspirin had higher odds of developing CRC than never-users. This could partly be explained by the time of data collection; the aspirin use could have been initiated as a secondary prevention after CRC diagnosis was given, leading to a larger proportion of aspirin-users in the CRC cohort. However, the indication for aspirin-use was not collected and is therefore not known.

Another important finding of this study is the difference between LS genotypes amongst patients with CRC detection before and during surveillance. The results show that MLH1 and MSH2 carriers have

TABLE 3B Univariable logistic regression and Fisher's exact test on patient characteristics of CRC cohort before surveillance and after index.

Variable	Before surveillance (n=80) n (%)	After index (n=18) n (%)	Crude OR (95% CI)	*p*
Sex				
Female	36 (84)	7 (16)	Ref	Ref
Male	44 (80)	11 (20)	0.92 (0.39-2.18)	0.85
Deceased	5 (63)	3 (37)		
Age at death (mean ± SD)	66.6 ± 17.0	69.7 ± 13.3		
Genotype**				
MLH1	32 (70)	14 (30)		<0.001*
MSH2	28 (88)	4 (12)		<0.001*
MSH6	12 (100)	0 (0)		Ref
PMS2	6 (100)	0 (0)		Ref
EPCAM	1 (100)	0 (0)		Ref
Mixed genotype	1 (100)	0 (0)		Ref
Age at LS diagnosis (mean ± SD)	51.4 ± 13.1	41.9 ± 12.1	0.95 (0.91–0.98)	0.05*
Smoking status				
Current smoker	9 (75)	3 (25)	1.90 (0.57-6.37)	0.30
Previous smoker	33 (87)	5 (13)	0.78 (0.29-2.05)	0.61
Never-smoker	37 (79)	10 (21)	Ref	Ref
Missing data	1 (100)	0 (0)		
BMI (mean ± SD)	26.1 ± 4.5	26.5 ± 5.7	1.03 (0.95-1.12)	0.45
Age at CRC diagnosis (mean ± SD)	44.9 ± 12.3	49.3 ± 15.1	1.02 (0.98-1.05)	0.35

Data are presented as numbers and percentages for nominal variables and as mean values and standard deviations for continuous variables. BMI, Body Mass Index; CI, Confidence Interval; OR, Odds Ratio; Ref, Reference Variable; SD, Standard Deviation.
*Statistically significant values are marked with an asterisk.
** MLH1 and MSH2 compared to MSH6, PMS2, EPCAM and mixed genotype using Fisher's exact test.

higher odds of CRC development during surveillance than MSH6, PMS2, and EPCAM carriers and patients with a mixed genotype. In accordance with these results, other studies have found that MLH1 and MSH2 carriers have a higher risk of developing CRC than MLH6 carriers (25), as well as PMS2 carriers (14–16), which could also be explained by the more rapid CRC development amongst MLH1 and MSH2 carriers (6). This finding could be used to bring forward a more individualized approach towards LS surveillance in which genotype is taken into consideration when an appropriate surveillance interval is recommended, which has previously been proposed by Goverde et al. (26).

This study is conducted on the outcome of colonoscopy surveillance amongst LS patients with CRC data, as well as structured data on individual factors, which are compared amongst patients with CRC detection before and during surveillance. Other studies, such as that conducted by Engel et al. (6), have investigated the optimal surveillance interval amongst LS patients but were unable to take individual risk factors into account.

TABLE 4 Distribution of TNM classification between CRC detected before and during surveillance.

	Before surveillance	During surveillance		
TNM stage	n (%)	At index n (%)	After index n (%)	Total, n (%)
Stage I	17 (21)	8 (80)	9 (50)	34 (31)
Stage II	20 (25)	2 (20)	3 (17)	25 (23)
Stage III	21 (26)	0 (0)	3 (17)	24 (22)
Stage IV	2 (3)	0 (0)	2 (11)	4 (4)
Missing data	20 (25)	0 (0)	1 (6)	21 (19)
Total	80 (100)	10 (100)	18 (100)	108 (100)

Data are presented as numbers (n) and percentages (%).

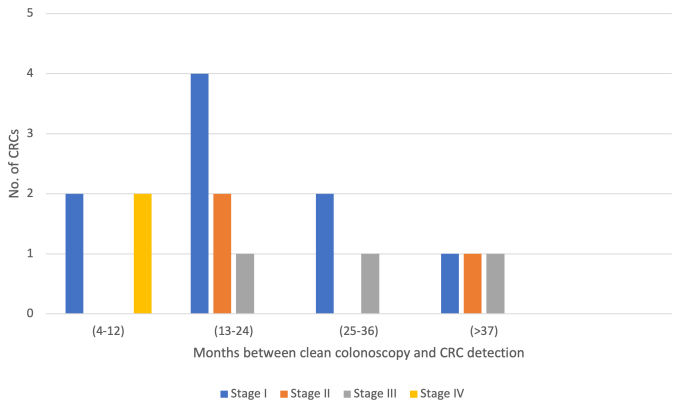


FIGURE 1
Colorectal cancer (CRC) detection after index. Intervals between clean colonoscopies and CRC detection of TNM-classified CRCs detected after index.

A limitation of this study is the fact that it is a single-center study with results from one hospital only. Since genetic testing in Stockholm is only performed at the Karolinska University Hospital, however, most of the LS patients had their follow-up at this hospital as well. Another limitation of this study is the lack of quantification within the "smoking status" variable. It could be of benefit to collect data about smoking in pack-years to have clearer definitions within the categories of smokers. However, since our results show a significant increase of CRC development amongst current and previous smokers, this is an important finding to bring forward.

Another limitation of this study is that it revolves around quantitative data on colonoscopies, but does not take qualitative data into consideration. Lappalainen et al. suggested in their study that the quality of colonoscopy is usually not correlated with incident CRCs in LS (27). However, it could be of importance to take the quality of colonoscopies into account when investigating an optimal surveillance interval; the adenoma detection rate and cleanliness of bowel are important since incomplete colonoscopies might cause a delay in CRC detection. Therefore, the quality of the surveillance endoscopies performed in our cohort needs to be studied further.

In conclusion, we found that 35% of the CRC cases detected during surveillance were found after the ESGE's recommended interval of 24 months (17). MLH1 and MSH2 carriers were at higher risk of developing CRC during surveillance. Additionally, men, current or previous smokers, and patients with a higher BMI were at higher risk of developing CRC. Currently, LS patients are recommended a "one-size-fits-all" colonoscopy surveillance program. The present results, however, support the development of a risk score in which individual risk factors, such as sex, genotype, smoking, and BMI, should be taken into consideration when identifying LS patients that may benefit from an annual surveillance program and individuals at low risk for whom frequency of surveillance may be reduced.

Data availability statement

The data sets presented in this article are not readily available. The data set is still under construction for research purposes. Requests to access the data sets should be directed to ann-sofie.backman@ki.se.

Author contributions

Study concept and design: A-SB. Acquisition of data: SB, NJ, JB, AH, and A-SB. Statistical analysis: NJ and AA. Drafting of the manuscript: NJ, AA, AF, and A-SB. All authors contributed to the article and approved the submitted version.

Funding

This study was supported with funding from Karolinska Institutet, Bengt Ihre's Research Foundation (SLS-593871), The Swedish Research Council (Dnr 2017-00557), and The Cancer Foundation in Sweden (nr 21 1500 Fk).

Acknowledgments

Special thanks to Prof. Annika Lindblom who began the research on LS patients at Karolinska University Hospital in Stockholm, Sweden, and laid the groundwork for this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Colorectal cancer awareness month. (2022). International Agency for Research on Cancer. Available at: https://www.iarc.who.int/featured-news/colorectal-cancer-awareness-month-2022/.

2. Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B, et al. Meta-analyses of colorectal cancer risk factors. *Cancer Causes Control* (2013) 24(6):1207–22. doi: 10.1007/s10552-013-0201-5

3. Mao R, Krautscheid P, Graham RP, Ganguly A, Shankar S, Ferber M, et al. Erratum to: ACMG technical standards and guidelines for genetic testing for inherited colorectal cancer (Lynch syndrome, familial adenomatous polyposis, and MYH-associated polyposis) (Genetics in medicine, (2014), 16, 1, (101-116), 10.1038/gim.2013.166). *Genet Med* (2021) 23(10):1807–17. doi: 10.1038/s41436-021-01207-9

4. Voskuil DW, Vasen HFA, Kampman E, Van't Veer P. Colorectal cancer risk in HNPCC families: Development during lifetime and in successive generations. *J Cancer Wiley-Liss Inc* (1997) 72:205–9. doi: 10.1002/(SICI)1097-0215(19970717)72:2<205::AID-IJC1>3.0.CO;2-V

5. Biller LH, Syngal S, Yurgelun MB. Recent advances in lynch syndrome. In: *Familial cancer*, vol. 18. Springer Netherlands (2019). p. 211–9.

6. Engel C, Vasen HF, Seppälä T, Aretz S, Bigirwamungu-Bargeman M, de Boer SY, et al. No difference in colorectal cancer incidence or stage at detection by colonoscopy among 3 countries with different lynch syndrome surveillance policies. *Gastroenterology* (2018) 155(5):1400–1409.e2. doi: 10.1053/j.gastro.2018.07.030

7. Lewandowska A, Rudzki G, Lewandowski T, Stryjkowska-Góra A, Rudzki S. Title: Risk factors for the diagnosis of colorectal cancer. *Cancer Control* (2022) 29. doi: 10.1177/10732748211056692

8. Lauby-Secretan B, Vilahur N, Bianchini F, Guha N, Straif K. The IARC perspective on colorectal cancer screening. *N Engl J Med* (2018) 2018:1734–40. doi: 10.1056/NEJMsr1714643

9. Pande M, Lynch PM, Hopper JL, Jenkins MA, Gallinger S, Haile RW, et al. Smoking and colorectal cancer in lynch syndrome: Results from the colon cancer family registry and the university of Texas M.D. Anderson cancer center. *Clin Cancer Res* (2010) 16(4):1331–9. doi: 10.1158/1078-0432.CCR-09-1877

10. Watson P, Ashwathnarayan R, Lynch HT, Roy HK. Tobacco use and increased colorectal cancer risk in patients with hereditary nonpolyposis colorectal cancer (Lynch syndrome). *Arch Intern Med* (2004) 164(22):2429–31. doi: 10.1001/archinte.164.22.2429

11. Lazzeroni M, Bellerba F, Calvello M, Macrae F, Win AK, Jenkins M, et al. A meta-analysis of obesity and risk of colorectal cancer in patients with lynch syndrome: The impact of sex and genetics. *Nutrients* (2021) 13(5):1736. doi: 10.3390/nu13051736

12. Sievänen T, Törmäkangas T, Laakkonen EK, Mecklin JP, Pylvänäinen K, Seppälä TT, et al. Body weight, physical activity, and risk of cancer in lynch syndrome. *Cancers (Basel)* (2021) 13(8):1849. doi: 10.3390/cancers13081849

13. Botma A, Nagengast FM, Braem MGM, Hendriks JCM, Kleibeuker JH, Vasen HFA, et al. Body mass index increases risk of colorectal adenomas in men with lynch syndrome: The GEOLynch cohort study. *J Clin Oncol* (2010) 28(28):4346–53. doi: 10.1200/JCO.2010.28.0453

14. Goecke T, Schulmann K, Engel C, Holinski-Feder E, Pagenstecher C, Schackert HK, et al. Genotype-phenotype comparison of German MLH1 and MSH2 mutation carriers clinically affected with lynch syndrome: A report by the German HNPCC consortium. *J Clin Oncol* (2006) 24(26):4285–92. doi: 10.1200/JCO.2005.03.7333

15. Senter L, Clendenning M, Sotamaa K, Hampel H, Green J, Potter JD, et al. The clinical phenotype of lynch syndrome due to germ-line PMS2 mutations. *Gastroenterology* (2008) 135(2):419–28. doi: 10.1053/j.gastro.2008.04.026

16. Lamba M, Wakeman C, Ebel R, Hamilton S, Frampton C, Kiesanowski M, et al. Associations between mutations in MSH6 and PMS2 and risk of surveillance-detected colorectal cancer. *Clin Gastroenterol Hepatol* (2020) 18(12):2768–74. doi: 10.1016/j.cgh.2020.03.048

17. van Leerdam ME, Roos VH, van Hooft JE, Balaguer F, Dekker E, Kaminski MF, et al. Endoscopic management of lynch syndrome and of familial risk of colorectal cancer: European society of gastrointestinal endoscopy (ESGE) guideline. *Endoscopy Georg Thieme Verlag* (2019) 51:1082–93. doi: 10.1055/a-1016-4977

18. Bernstedt SW, Björk J, Fritzell K, Spigelman AD, Björck E, Backman AS. Room for improvement: One third of lynch syndrome patients presenting for genetic testing in a highly specialised centre in Stockholm already have cancer. *Hered Cancer Clin Pract* (2021) 19(1):18. doi: 10.1186/s13053-021-00171-4

19. de Jong AE, Hendriks YMC, Kleibeuker JH, de Boer SY, Cats A, Griffioen G, et al. Decrease in mortality in lynch syndrome families because of surveillance. *Gastroenterology.* (2006) 130(3):665–71. doi: 10.1053/j.gastro.2005.11.032

20. Thompson BA, Spurdle AB, Plazzer JP, Greenblatt MS, Akagi K, Al-Mulla F, et al. Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the InSiGHT locus-specific database. *Nat Genet* (2014) 46:107–15. doi: 10.1038/ng.2854

21. Engel C, Rahner N, Schulmann K, Holinski-Feder E, Goecke TO, Schackert HK, et al. Efficacy of annual colonoscopic surveillance in individuals with hereditary nonpolyposis colorectal cancer. *Clin Gastroenterol Hepatol* (2010) 8(2):174–82. doi: 10.1016/j.cgh.2009.10.003

22. Burn J, Sheth H, Elliott F, Reed L, Macrae F, Mecklin JP, 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. *Clin Gastroenterol Hepatol* (2020) 395(10240):1855–63. doi: 10.1016/S0140-6736(20)30366-4

23. Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. *n Engl J Med* (2003) 348:883–90. doi: 10.1056/NEJMoa021633

24. Logan RFA, Grainge MJ, Shepherd VC, Armitage NC, Muir KR. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. *Gastroenterology.* (2008) 134(1):29–38. doi: 10.1053/j.gastro.2007.10.014

25. Vasen HFA, Abdirahman M, Brohet R, Langers AMJ, Kleibeuker JH, van Kouwen M, et al. One to 2-year surveillance intervals reduce risk of colorectal cancer in families with lynch syndrome. *Gastroenterology* (2010) 138(7):2300–6. doi: 10.1053/j.gastro.2010.02.053

26. Goverde A, Eikenboom EL, Viskil EL, Bruno MJ, Doukas M, Dinjens WNM, et al. Yield of lynch syndrome surveillance for patients with pathogenic variants in DNA mismatch repair genes. clinical gastroenterology and hepatology. (2020) 18(5):1112–1120.e1. doi: 10.1016/j.cgh.2019.08.043

27. Lappalainen J, Holmström D, Lepistö A, Saarnio J, Mecklin JP, Seppälä T. Incident colorectal cancer in lynch syndrome is usually not preceded by compromised quality of colonoscopy. *Scand J Gastroenterol* (2019) 54(12):1473–80. doi: 10.1080/00365521.2019.1698651

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 21 February 2023
DOI 10.3389/fonc.2023.1127329

Check for updates

OPEN ACCESS

EDITED BY
Inge Bernstein,
Aalborg University Hospital, Denmark

REVIEWED BY
Lone Sunde,
Aalborg University Hospital, Denmark
Elke Holinski-Feder,
Medical Genetic Center (MGZ), Germany
Päivi Peltomäki,
University of Helsinki, Finland

*CORRESPONDENCE
Sanne W. Bajwa–ten Broeke
s.w.ten.broeke@umcg.nl

SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 19 December 2022
ACCEPTED 01 February 2023
PUBLISHED 21 February 2023

CITATION
Andini KD, Nielsen M, Suerink M, Helderman NC, Koornstra JJ, Ahadova A, Kloor M, Mourits MJE, Kok K, Sijmons RH and Bajwa-ten Broeke SW (2023) PMS2-associated Lynch syndrome: Past, present and future.
Front. Oncol. 13:1127329.
doi: 10.3389/fonc.2023.1127329

COPYRIGHT
© 2023 Andini, Nielsen, Suerink, Helderman, Koornstra, Ahadova, Kloor, Mourits, Kok, Sijmons and Bajwa-ten Broeke. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

PMS2-associated Lynch syndrome: Past, present and future

Katarina D. Andini[1], Maartje Nielsen[2], Manon Suerink[2], Noah C. Helderman[1], Jan Jacob Koornstra[3], Aysel Ahadova[4], Matthias Kloor[4], Marian J.E. Mourits[5], Klaas Kok[1], Rolf H. Sijmons[1] and Sanne W. Bajwa–ten Broeke[1]*

[1]Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, Netherlands, [2]Department of Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands, [3]Department of Gastroenterology and Hepatology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands, [4]Department of Applied Tumour Biology, Institute of Pathology, Heidelberg University Hospital, and Clinical Cooperation Unit Applied Tumor Biology, German Cancer Research Center, Heidelberg, Germany, [5]Department of Gynaecology, University Medical Center Groningen, University of Groningen, Groningen, Netherlands

Carriers of any pathogenic variant in one of the MMR genes (*path_MMR* carriers) were traditionally thought to be at comparable risk of developing a range of different malignancies, foremost colorectal cancer (CRC) and endometrial cancer. However, it is now widely accepted that their cancer risk and cancer spectrum range notably depending on which MMR gene is affected. Moreover, there is increasing evidence that the MMR gene affected also influences the molecular pathogenesis of Lynch syndrome CRC. Although substantial progress has been made over the past decade in understanding these differences, many questions remain unanswered, especially pertaining to *path_PMS2* carriers. Recent findings show that, while the cancer risk is relatively low, PMS2-deficient CRCs tend to show more aggressive behaviour and have a worse prognosis than other MMR-deficient CRCs. This, together with lower intratumoral immune infiltration, suggests that PMS2-deficient CRCs might have more in common biologically with sporadic MMR-proficient CRCs than with other MMR-deficient CRCs. These findings could have important consequences for surveillance, chemoprevention and therapeutic strategies (e.g. vaccines). In this review we discuss the current knowledge, current (clinical) challenges and knowledge gaps that should be targeted by future studies.

KEYWORDS

Lynch syndrome (hereditary nonpolyposis colorectal cancer), mismatch repair (MMR), PMS2 gene, colorectal cancer, endometrial cancer, carcinonogenesis

Introduction

It has long been thought that germline pathogenic *PMS2* variant carriers (*path_PMS2* carriers) represent only a small minority of Lynch syndrome (LS) patients. However, more recent investigations have revealed that the population frequency of *path_PMS2* carriers is actually the highest among the four mismatch repair (MMR) genes (1 in 714) (1). Germline *path_PMS2* carriers have a much lower risk of developing cancer compared to other *path_MMR* carriers, although the cancer risk seems to vary widely among affected individuals from the same family (2–4). Stratifying LS patients by MMR gene is therefore of vital importance for research and clinical purposes. Indeed, accumulating evidence suggests that PMS2-deficient (dPMS2) tumours show distinct biological behaviour that differs from other MMR-deficient (dMMR) cancers. At the moment, it is still unknown whether the preventive measures now being investigated, such as vaccination or aspirin chemoprevention, would benefit *path_PMS2* carriers in the same way as other *path_MMR* carriers. Given these clinical and research questions, we set out to review the literature on these topics and challenges, discuss clinical scenarios that highlight their importance and identify knowledge gaps to be addressed by future studies.

Past

Identification of path_PMS2 carriers

The clinical involvement of *path_PMS2* variants in LS was first described in 1994. However, clinical testing of the gene did not become available until 2009 because the PMS2 gene is notoriously difficult to analyse due to the existence of multiple pseudogenes (5–9). *PMS2* is located on the short arm of chromosome 7 and spans 15 exons. Multiple regions with over 90% homology have been identified, all on chromosome 7, and these pseudogene regions make interpretation of sequencing results of the *PMS2* gene challenging. A variety of strategies, including designing long-range amplicons (9) and RNA analysis (7), have helped overcome this problem and led to improved variant detection and increased identification of *path_PMS2* carriers. Another explanation for the reported underestimation of *PMS2*-LS prevalence lies in selection of families for genetic testing using family history or age of diagnosis (Bethesda and Amsterdam criteria) (10). Previous work has shown that *path_PMS2* variants are predominantly found in families that do not fulfil these criteria (2, 4, 11, 12).

The traditional approach to identifying LS using clinical selection criteria is of limited use in the identification of *path_PMS2* carriers, making it difficult to determine their prevalence. Answers can be sought in population-based colorectal cancer (CRC) cohorts. Studies using immunohistochemical staining (IHC) in CRCs from population-based cohorts have shown that isolated PMS2 loss of expression, indicative of *path_PMS2* variants, is present in 0.5–1.5% of unselected CRCs (2, 12). The fraction of isolated PMS2 loss in MSI CRCs varies between 1–8% (13–15). More than half of such tumours have been shown to be caused by a germline *path_PMS2* variant (16, 17). Another possibility is the presence of double somatic hits, reported to be the cause in 13-17% of isolated dPMS2-CRC (17). Of note, this fraction is lower than for tumours with MLH1/PMS2 or MSH2/MSH6 loss. In other words, isolated PMS2 loss is highly indicative of a germline *path_PMS2* variant. One study of population-based CRCs found a higher percentage of isolated PMS2 than of MSH2 loss of expression (12% versus 11%) in tumours with negative MMR staining (12). Moreover, recent studies have also shown that *PMS2* and *MSH6* variants are much more prevalent in unselected (population-based) cohorts than in those selected by traditional family history criteria. Estimates of (Western) population carrier frequency based on statistical approaches are 1 in 714 for *PMS2* and 1 in 758 for *MSH6*, whereas the prevalences are 1 in 1946 for *MLH1* and 1 in 2841 for *MSH2* (1). Secondly, an unselected study involving the entire Icelandic population found an incidence of 1 in 226 for *PMS2* and *MSH6* variants combined (18). Another finding that may indicate that the carrier frequency of *PMS2* variants is higher than that of *MLH1* and *MSH2* variants involves the fact that biallelic *path_PMS2* variants comprise more than half of the homozygous or compound heterozygous variants in patients reported with the rare early-onset autosomal recessive disorder Constitutional MMR Deficiency (CMMR-D) (31/57) (19). However, this may also result from the lower penetrance of *path_PMS2* variants, which makes them difficult to detect by clinical selection criteria, as will be discussed in the next section. In such a situation, fetuses with biallelic *path_MLH1* or *path_MSH2* variants might be less viable than fetuses with biallelic *path_PMS2* variants, leading to overrepresentation of biallelic *path_PMS2* carriers amongst CMMR-D cases. Of note, the possible occurrence of a child with CMMR-D in *path_PMS2* families, especially consanguineous ones, is an argument for the importance of detecting *path_PMS2* carriers despite the relatively low penetrance.

The most significant improvement in the detection of *path_MMR* carriers is most likely introduction of universal IHC staining of the MMR protein. While the specific screening strategy differs by country, ranging from true universal screening of all CRCs to age-dependent IHC, this approach has proven to be cost-effective and has the added benefit that no additional selection criteria are needed (20–22).

Cancer risks

The first large cohort of *path_PMS2* carriers (55 index patients and 55 relatives) was reported by Senter et al. in 2008 (2). They reported a cumulative risk for CRC at age 70 years of 20% (95% confidence interval (CI): 11–34%) for male *path_PMS2* carriers and 15% (95% CI: 8–26%) for female *path_PMS2* carriers. The cumulative risk at age 70 for endometrial cancer (EC) was found to be 15% (23). These risks are substantially lower than those previously reported for *path_MLH1*, *path_MSH2* and *path_MSH6* carriers, which range from 25–75% up to age 70 years for CRC and 30–35% for EC.

In 2015, we analysed 98 PMS2 families and found similar cancer risks to those previously reported, i.e. risks of 11–19% for CRC and 12% for EC up to age 70 years (Table 1) (4), further supporting that PMS2-LS patients face significantly lower risks than other LS patients. This study was underpowered for analyses of less frequent LS-associated cancers. In a second, larger study by our group, consisting of 284 families and providing enough power to also

estimate extra-colonic and extra-EC risks, we found increased risk only for CRC and EC (3). The cumulative risks for CRC and EC could be estimated up to age 80 years and were 13–14% and 14%, respectively. Statistically, the cumulative risks for ovarian, gastric, hepatobiliary, bladder, renal, brain, breast, prostate, or small bowel cancer did not significantly deviate from risks in the general population (3). Cancer risks for *path_PMS2* carriers compared to the general population and other *path_MMR* carriers are given in Table 1.

All three of the studies described above used modified segregation analysis to correct for ascertainment bias. This form of bias is the selection of families with relatively high penetrance due to selection criteria, most likely resulting in overestimation of cancer risk (24). However, retrospective analyses have been important because they estimate risk without surveillance. But there are other ways to deal with ascertainment bias besides modified segregation analysis. The first is to include families that have not been ascertained because of the LS phenotype, a group that includes those ascertained through universal IHC for the MMR proteins. These families usually exhibit much milder phenotypes compared to clinically ascertained families. A recent study published on MedRxiv reported much lower cancer risks and families ascertained through population screening in comparison to clinical ascertainment, namely 15.2% vs. 27.1% for *path_MLH1* and 3.2% vs. 25.2% for *path_MSH2*, respectively (25). Theoretically this could mean that *path_PMS2* carriers ascertained from the population or as incidental findings would only be at population risk (see also the clinical challenges section in this review). Cancer risks established in this way would be of high value considering the occurrence of *path_PMS2* variant detection as incidental findings for example (see case discussions below). However, such cases mostly remained unidentified before the introduction of universal screening and to our knowledge such data is currently unavailable in sufficient quantities to estimate these risks.

Ascertainment bias may also be circumvented by analysing families ascertained because of a patient with CMMR-D. These patients carry homozygous or compound heterozygous variants in one of the MMR genes, usually *PMS2* or *MSH6*. Due to their constitutional dMMR, these patients display a very striking phenotype of cancer in childhood. They also present with axillary freckling and café-au-lait macules (19, 26–29). As *de novo* MMR variants have been reported to be extremely rare, parents are usually carriers of a heterozygous MMR variant, and therefore have LS. Notably, these families almost never meet traditional selection criteria due to a very mild phenotype. Our group has gathered a large cohort of CMMR-D-ascertained LS families and found similar results to the previously published cancer risks, 8.7% (95% CI 4.3–

TABLE 1 Overview of reported cancer risks.

	General population (lifetime)	Lynch syndrome Barrow et al (up to age 70) *	PMS2-associated Lynch syndrome					
			Senter et al 2008 (95% CI)	Ten Broeke et al 2015 (95% CI)	Ten Broeke et al 2018		Moller et al	
					Up to age 70 (95% CI)	Up to age 80 (95% CI)	IMRC (retrospective)	PLSD (prospective)
Colorectal	~4–6%	25–75%	♂: 20% (11–34%)	♂: 19% (6–30%)	♂: 6% (3–13%)	♂: 13% (8–22%)	♂: 7% (6–8%) ♀: 6 (5–6%)	♂:11% (3–37) ♀: 8 (2–29%)
			♀: 15% (8–26%)	♀: 11% (2–18%)	♀: 6% (3–12%)	♀: 12% (7–21%)		
Endometrial	~3%	30–35%	15% (6–35%)	12% (3–20%)	10% (5–17%)	14% (7–24%)	N/A	
Ovarian	~1%	6–14 %	N/A	SIR: 12.0 (3.3–30.7)	HR: 1.52 (0.45–5.05)			
Gastric	~1%	0.7–13 %		SIR: 0.0 (0–6.5)	HR: 2.07 (0.73–5.87)			
Urothelial	~1–2%	1.9–11.2%		SIR (bladder): 2.0 (0.05–11.2)	HR: 2.05 (0.77–5.45) (kidney and ureter)			
				SIR (renal pelvis): 50.5 (6.1–182.4)				
Small Bowel	~0.1%	0.6–7%		SIR: 118.9 (38.6–277.4)	Too few events for analysis			
CNS	~0.5%	1.2–3.7%		SIR: 2.7 (0.069–15.2)	HR: 2.09 (0.79–5.54) (brain)			
Pancreas & biliary tree	~1–2%	0.6–2.1%		SIR: 0 (0–12) (only pancreas)	HR: 1.02 (0.12–8.60) (hepatobiliary)			
Breast	~12%	Conflicting results of association		SIR: 3.8 (1.9–6.8)	HR: 1.30 (0.79–2.16)			

SIR: Standardized Incidence Ratio. HR: Hazard Ratio. 95% CI: Confidence Interval.
IMRC: International Mismatch Consortium. PLSD: Prospective Lynch Syndrome Database.
*path_MLH1, path_MSH2 and path_MSH6 combined.

12.7%) up to age 70 years for both sexes combined (30), which confirms previous reports of low cancer risks for *PMS2*-LS patients. The great advantage of this method is that it provides cancer risks similar to the retrospective approach, i.e. risks without surveillance, but without ascertainment bias of families selected based on the LS phenotype. However, due to the rarity of families with a CMMR-D case the amount of families that can be included is much lower.

A third method of estimating cancer risks is to gather prospective data, and a global collaboration has now been formed to gather such data. The Prospective LS Database (PLSD) has currently published multiple reports on penetrance (23, 31–33). Their most recent study estimated risks of 11% (95% CI: 3–37%) and 8% (95% CI: 2–29%) for *path_PMS2* carriers up to age 70 for men and women, respectively (32). Cancer risk estimates from the PLSD are aimed at determining the risks while under surveillance, which is important information when counselling patients. However, comparison of these risks to retrospective studies are difficult due to the inherent differences of these approaches. For a further discussion on this we refer to the section on clinical guidelines.

Lastly, it is well known that cancer penetrance seems to vary between and within families. One study that attempted to capture this variation by estimating the proportion of carriers that were at a specific risk found that most *path_PMS2* carriers had a cumulative risk lower than 20%. However, a small fraction of carriers were still at very high risk (more than 80%) (34). It is likely that other (strong) risk factors play a role in these carriers and/or families, as discussed in more detail below.

Genotype–phenotype correlations

One factor that could influence penetrance is the specific variant present. Whether or not cancer penetrance in LS patients is dependent on the specific type of pathogenic variant identified in an MMR gene is still a subject of debate. It is conceivable that variants that lead to a partially functional protein could explain more mildly affected families. However, a recent PLSD report found no differences in penetrance between missense or truncating variants in *path_MLH1* or *path_MSH2* carriers (35). Missense and truncating mutations make up the majority of reported *path_MLH1* (both 40%), *path_MSH2* (31% and 49%, respectively), and *path_MSH6* (49% and 43%, respectively) variants. In contrast, missense mutations make up 62% of *path_PMS2* variants, considerably higher than the percentage of truncating mutations (24%) (36). There is one study of European *path_PMS2* carriers that identified a difference of 9 years delay in mean age at first CRC diagnoses for variants that had retained RNA expression (37). Based on this study, most of the variants discovered were categorized as missense variants causing the loss of *PMS2* mRNA expression. There were also several missense variants that did not seem to impact *PMS2* mRNA expression (i.e. c.137G>T (p.Ser46Ile) and c.2113G>A (p.Glu705Lys)), which resulted in residual function of PMS2 protein. The residual protein function might explain the fact that this group of patients develop CRC at older age compared to the group bearing variants affecting mRNA expression (51.1 years vs. 60 years, respectively). Of note, effects on RNA expression could not be taken into account in the PLSD study because these data were not available. So whether these findings can also be extrapolated to other MMR genes remains to be determined. Interestingly, a report on CMMR-D patients carrying a biallelic NM_000535.5:c.2002A>G (p.Ile668Val) variant described an attenuated phenotype where the age at first cancer was strikingly different, namely 22 years for carriers of this variant versus 8 years for truncating *PMS2* variants (38). Functional studies in these patients showed they retained full-length protein in normal tissue. These findings, if replicated, could have important consequences for clinical risk stratification and even surveillance guidelines.

Molecular pathways of dMMR-associated carcinogenesis

In healthy individuals, MMR proteins function as heterodimers in two main complexes consisting of (1) MutS homologues MSH2 and either MSH6 or MSH3 and (2) MutL homologues MLH1 binding to PMS2, PMS1 or MLH3. The MutS complex recognises a mismatch between the opposing DNA strands and recruits the MutL complex, which then initiates repair. These complexes act together in repairing mismatches of single nucleotides and insertion-deletion loops (5, 36, 39). The theory regarding the lower penetrance of *path_PMS2* variants is that MLH1/MLH3 and/or MLH1/PMS1 heterodimers can partially compensate for the loss of the MLH1/PMS2 heterodimer. Indeed, *Pms2* -/- and *Mlh3* -/- mice have a similar mutational load and disease progression to *Mlh1* -/- mice, suggesting this is a plausible explanation (40).

Tumours in *path_MMR* carriers arise or progress when the remaining wild type *MLH1*, *MSH2*, *MSH6*, or *PMS2* allele is deactivated because of a second hit, in line with Knudson's "Two-hit" hypothesis (41). This leads to impaired MMR and subsequent accumulation of somatic variants in other (cancer) genes, which can eventually lead to uncontrolled cell growth and cancer. Hallmarks of these tumours are the absence of MMR protein expression by IHC and, as a result of faulty MMR, the shortening and lengthening of regions with nucleotide repeats. These regions, which are common in our genome, can exist inside and outside protein coding regions and are referred to as microsatellites, with changes in their length referred to as microsatellite instability (MSI). Although these changes can also be caused by somatic pathogenic MMR gene variants when they hit both alleles of a MMR gene, they are very helpful in the selection of patients for germline DNA testing for LS (42).

Testing for MSI, somatic variants caused by MSI, or other mechanisms and loss of MMR by IHC staining also plays a role in the study of LS carcinogenesis. Recent studies have proposed three distinct carcinogenesis pathways in LS (Figure 1) (43, 44). These are:

Pathway 1 – the traditional proficient MMR (pMMR) adenoma to dMMR CRC pathway,

Pathway 2 – a combined pathway where dMMR adenomas grow from dMMR crypts proceeding to dMMR CRC and

Pathway 3 – a more recently discovered pathway where dMMR CRC develops directly from morphologically normal dMMR crypt foci (MMR-DCF).

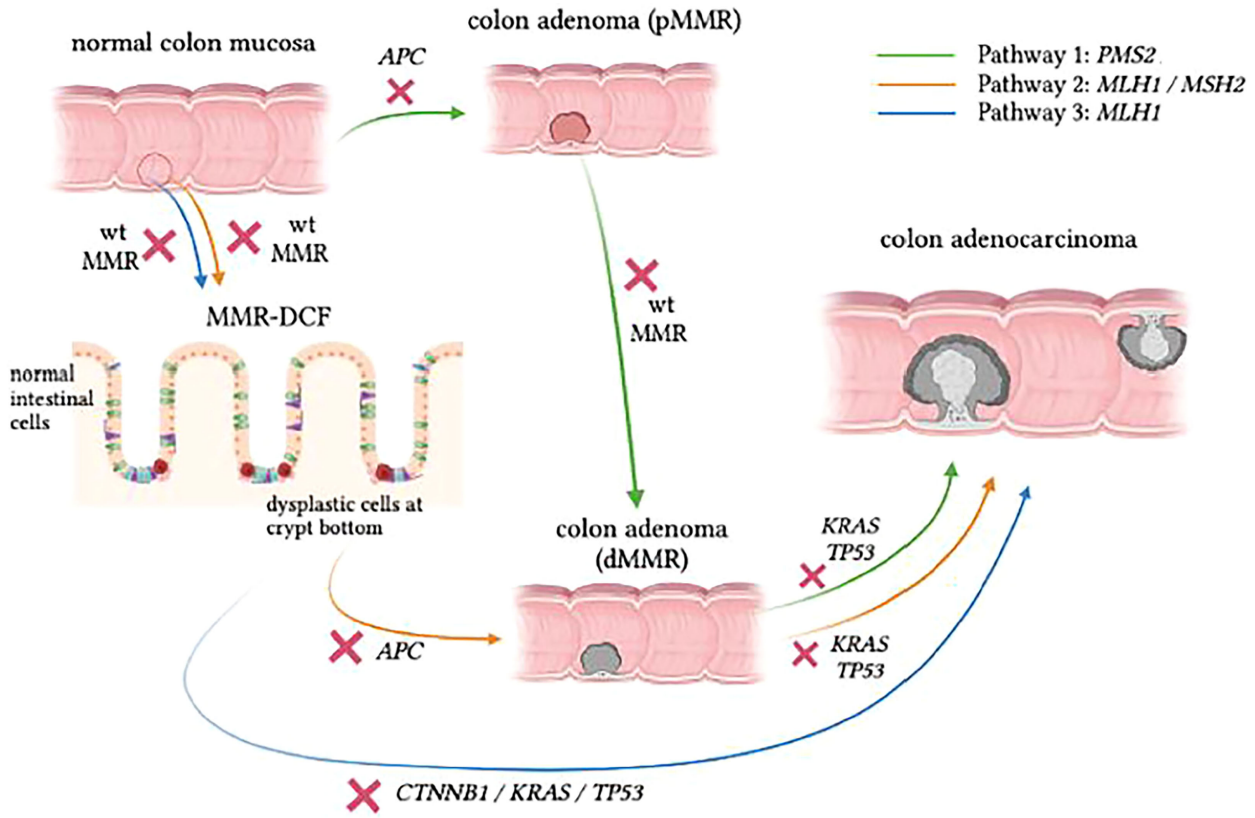


FIGURE 1
Proposed pathways of CRC development in LS. Pathway 1 (green) shows similarities to the classic adenoma-carcinoma sequence, but the progression speed is accelerated in LS carriers. Pathway 2 (orange) follows the pattern of adenoma formation from MMR-DCF, which eventually gives rise to adenocarcinoma as a consequence of accumulating somatic mutations with absent MMR activity in the background . In Pathway 3 (blue), which is rather insidious, dMMR LS-CRC can skip the adenomatous phase to grow directly into the colonic wall. Figure based on data from Ahadova et al., 2018[42] and Engel et al., 2020[68].

About a decade ago, IHC staining of colon specimens from LS patients revealed areas of morphologically normal mucosa that were already devoid of MMR protein, which demonstrated early loss of MMR function in otherwise normal-looking mucosa (45). These areas are referred to as mismatch repair deficient crypt foci (MMR-DCF), and they are considered unique to LS because they are rarely found (<1%) in biopsy specimens of normal mucosa in the vicinity of sporadic MSI-high tumours (45). In addition, MMR-DCF were more frequently found in colon tissue than in small intestine in LS patients, and their abundance might increase with age (46). Most likely, a large percentage of LS-CRC cases develop through MMR-DCF that continuously accumulate variants, resulting in malignant transformation of colonic cells regardless of the presence of an adenoma, as indicated in Figure 1. In the past, somatic β-catenin variants have been linked to CRC formation from MMR-DCF (47). The finding that β-catenin variants have not been observed in PMS2-LS-CRC has led to the hypothesis that CRCs in these *path_PMS2* carriers developed through an adenoma precursor lesion and not directly from MMR-DCFs (48).

A possible explanation for the differences observed between *path_PMS2* carriers and other *path_MMR* carriers is that dPMS2 may only occur at a later stage of tumour development (Pathway 1). More information on the specific mutational spectrum of LS-tumours could help to further corroborate this. Somatic variants identified in LS-lesions often demonstrate an overrepresentation of C>T variants, which corresponds to mutational signature 6 associated with MMR deficiency (48, 49). Mutated genes in dMMR CRC include *APC*, *KRAS*, *CTNNB1* and *TGFBR2*. Somatic *APC* variants are assumed to occur after the loss of the wildtype MMR allele in the majority of LS-CRCs and to accelerate the malignant transformation in LS. However, although the majority of dysplastic LS adenomas are dMMR, it is important to note that some adenomas in LS do retain MMR capacity (pMMR adenomas), in accordance with Pathway 1 (43). It has also been suggested that dPMS2 CRC develops solely through pMMR adenomas, with dPMS2 occurring at a relatively late stage and not as an initiating event. Indeed, data from a previous study by our group reported a relatively low frequency of the somatic *KRAS* hotspot variants G12D and G13D that were previously associated with the mutational signature of MMR deficiency (48, 49). The lower frequency of these two variants in dPMS2 tumours, combined with the fact that *KRAS* variants are known to occur in a relatively late stage of tumour progression, has led to the hypothesis that loss of the wildtype *PMS2* allele is a secondary and not an initiating event in CRCs that develop in *path_PMS2* carriers. Future studies are needed to confirm this hypothesis and evaluate why dPMS2 predominantly contributes as a late event in LS carcinogenesis.

Another approach to investigate differences between molecular pathways in the different subgroups of *path_MMR* carriers is to study

the coding microsatellite instability (cMSI) spectrum (50). Our group therefore performed a second tumour study analysing 16 dPMS2 CRCs from confirmed *path_PMS2* carriers. The cMSI spectrum of dPMS2 CRCs did not show any significant differences from dMLH1/dMSH2 CRCs, even after correction for tumour stage. If confirmed by larger studies, this is an interesting finding from an immunological perspective. An important aspect of dMMR CRC is activation of the immune system. CRCs in *path_MMR* carriers are known to bear a significantly higher number of pathogenic variants compared to sporadic CRC. These lesions with high mutational burden produce a relatively large amount of tumour-specific neoantigen. In the case of LS-CRC, the neoantigens resulting from insertions and deletions occurring in tumour cells with MSI are caused by a shift of the translational reading frame, with the resulting neoantigens termed frameshift peptides (FSPs). The presence of FSPs on the cellular membrane can trigger the recruitment and functionality of immune cells that surround the tumour mass. Consequently, MSI tumours display a high degree of immune infiltration. This is considered to play a major role in the favourable prognosis of LS-tumours (23). However, we and others have observed significantly lower CD3-positive T cell infiltration in dPMS2-CRCs compared to other dMMR-CRCs. One study looked at 93 dPMS2-CRCs and observed higher odds of disease specific death compared to other dMMR-CRCs. A plausible explanation for this more aggressive behaviour may be the lower degree of immune activation. The same study speculated that a lower level of mutational neoantigens may underlie the limited T cell infiltration in dPMS2 tumours (51). As described above, our data did not provide any evidence for a decreased amount of cMSI–induced neoantigens in dPMS2 CRCs. Therefore other explanations should be considered, including alternative immune evasion strategies, similar to what is seen in sporadic CRC. Moreover, these findings may be compatible with the hypothesis that dPMS2 occurs later during tumour evolution.

How do these MMR gene-dependent pathways relate to EC? Recent work has shown the existence of dMMR nonneoplastic endometrial glands (52). These dMMR glands were not present in population controls, suggesting that they are a benign precursor for EC in LS patients. More studies are needed to determine whether there are multiple pathways leading to EC and whether or not there are differences between the MMR genes as well.

Clinical guidelines

Interestingly, the CRC risk reported for *path_PMS2* carriers is only two or maximum three times higher than the general population risk. Is that high enough to offer surveillance colonoscopy? In the Netherlands for example, surveillance would be indicated when the CRC risk exceeds the threshold of three times the general population risk. Does it therefore follow that *path_PMS2* carriers should not undergo any colonoscopic surveillance, at least in countries using these threshold levels? To answer this question, we can look at several lines of evidence.

Firstly, a recent study compared retrospective International Mismatch Repair Consortium (IMRC) and prospective cancer risks from the PLSD (32). Retrospective studies are aimed at determining the cancer risk without colonoscopy and polypectomy, while prospective studies include patients that undergo surveillance and therefore estimate risk despite colonoscopy. The retrospective CRC risks for *path_PMS2* carriers were very similar, 7% for men (95% CI: 6–8%) vs 6% for women (95% CI: 5–6%), while the prospective risk was 11% (95% CI: 3–37%) vs 8% (95% CI: 2–29%). However, before age 50, the prospective *path_PMS2* PLSD cohort appeared to have slightly lower CRC risk than the retrospective IMRC cohort, although this was not statistically significant. Of note, this was the opposite for carriers of other *path_MMR* variants, suggesting that colonoscopic surveillance does not prevent CRC in these carriers, but might in fact be effective for *path_PMS2* carriers. A possible explanation for this is that dPMS2 tumours are believed to predominantly progress from adenomas, which are clearly visible, while the other dMMR tumours may progress directly from MMR-DCF, which may be more difficult to detect during colonoscopies. In the future, larger studies should shed more light on whether this is a clinically relevant difference. This naturally has important implications for the determination of clinical guidelines.

Secondly, as discussed above, the role of dPMS2 in tumour development seems to be confirmed by molecular studies and could result in increased adenoma progression to CRC (50). For the moment, the clinical and molecular characteristics together support the existence of colonoscopy guidelines for *path_PMS2* carriers. Whether use of other screening measures such as the Fecal Immunochemical Test instead of colonoscopy might also lead to substantially (and acceptably) lower cancer risk needs further research.

Present

Challenges in clinical practice

Recent guidelines have made a clear distinction between the different MMR carriers (53–56). For *path_PMS2* carriers colonoscopic surveillance starts at age 35 rather than at age 25. The foundation for this being the substantially lower cancer risk and later age at onset of CRC compared to other *path_MMR* carriers, as discussed above. Indeed, recent studies have shown that raising the starting age is (very) cost-effective without leading to substantial differences in disease outcomes (57, 58). The European Hereditary Tumour Group guideline takes this one step further by extending the colonoscopy interval to 5 years from 1–2 years (56). Below we present and discuss five cases from our daily practice that highlight challenges in clinical management of *path_PMS2* carriers. The aim of presenting these cases is not to replace current clinical guidelines but to serve as an illustration and stimulate further discussion.

Case 1

The daughter of a 60-year-old woman who had died from endometrioid type ovarian cancer is referred for genetic testing. Unfortunately, tumour tissue from the mother is not available for sequencing and/or IHC. In line with current Dutch guidelines, the daughter is offered germline DNA testing of our ovarian cancer gene panel, which includes the MMR genes. A likely pathogenic germline

variant in the *PMS2* gene is identified. There is no personal or family history of EC or CRC.

Case discussion: We suggest counselling the daughter and explaining that there is no evidence for a direct association between pathogenic variants in this gene and ovarian cancer (see also Table 1). The penetrance of this variant is likely very low given that the family history mentions no LS tumours. We believe that the *path_PMS2* variant should therefore be considered an incidental finding and could have been inherited from the father or mother. The pros and cons of colonoscopic and endometrial surveillance should carefully be discussed with the daughter in light of the likely low penetrance. Unfortunately, cancer risk data in such instances are not yet available.

In our centre we have now excluded the *PMS2* gene from the ovarian cancer panel because of lack of evidence for an association. Germline testing of this gene in an ovarian cancer panel would, in our opinion, be opportunistic screening, i.e. aimed at finding genetic disease predisposition unrelated to the diagnostic question. Such screening is currently not offered to our patients who undergo diagnostic testing.

Case 2

A ten-year-old boy from two non-consanguineous healthy parents presents with severe developmental delay and dysmorphic features. A SNP array is performed, and a small paternal deletion identified that includes 7p22.1 where the *PMS2* gene is located. It is believed that this is not an explanation for the boy's developmental delay and whole exome sequencing will be performed next. There is no family history of cancer of any type and no consanguinity. Should the pathogenic deletion of *PMS2* be discussed as an incidental finding and cascade screening subsequently be offered?

Case discussion: Further policy in this case is naturally highly dependent on national guidelines and the specific informed consent given by the parents regarding incidental/secondary findings. In the Netherlands, the standard is to only report highly penetrant variants in genes with clinical actionability. Based on UK Biobank findings for *MLH1* and *MSH2* (25), we suspect that *path_PMS2* cancer risks in the general population are even lower than reported for *PMS2*-LS families and might fall well below the national threshold of three times the population CRC risk, therefore not warranting such surveillance. Nevertheless, these risk figures are unavailable, and the only national *PMS2* guideline available to us is that for identifying pathogenic variants in the setting of suspected LS, which recommends colonoscopic surveillance. The ACMG guidelines on reporting secondary findings in patients undergoing diagnostic testing *do* recommend actively looking at *PMS2* for pathogenic variants and reporting those even if unrelated to the disease for which the testing was initially done (59). Those guidelines, however, are often based on cancer risk studies biased by selection and ascertainment, and this holds true for *PMS2*. A last argument in favour of reporting the deletion in this setting could be the identification of couples at risk of conceiving a child with CMMR-D. However, to our knowledge in most countries genetic testing for *path_MMR* variants is not routinely offered to partners of *path_MMR* carriers who want to conceive. The exception being cases with known consanguinity or a higher chance of biallelic offspring (e.g. isolated populations). Clearly there are pros and cons for reporting our *PMS2* finding in such situations: the possible increased CRC risk on the one hand and the lack of knowledge of cancer risks associated with *path_PMS2* as secondary finding, which might in fact be low, on the other. In the end we decided to share these considerations with the parents in addition to discussing the burden of colonoscopy and the possible alternative of the national CRC screening through faecal occult blood testing.

Case 3

A 30-year-old woman is pre-symptomatically tested for the *path_PMS2* variant in her family and found to be positive. She requests a prophylactic hysterectomy and oophorectomy. There is no family history of ovarian cancer and EC.

Case discussion: We advise extreme restraint with respect to a prophylactic hysterectomy in this case. The cumulative risk of EC is approximately 12% (3). Moreover, as survival for these tumours is extremely high (23, 33), we believe there is no indication for prophylactic hysterectomy. Of note, the Manchester recommendations for the management of gynaecological cancer in LS involved patient representatives who felt that it should be considered an option despite these considerations (53). However, in the Netherlands, we currently actively advise against gynaecological preventive surgery in *path_PMS2* carriers.

Case 4

A 55-year-old man presents with a T3N0 CRC, with no family history of CRC or EC. Universal IHC for the MMR proteins shows loss of expression of the PMS2 protein. The surgeon refers the patient for priority counselling and genetic testing. Choice of a specific operating procedure (i.e. segmental or hemi-colectomy) is postponed until the genetic testing results are available.

Case discussion: Extended colectomy with ileosigmoidal/ileorectal anastomosis is preferable to standard resection for *path_MLH1* and *path_MSH2* carriers given the increased risk of developing a metachronous cancer after segmental colectomy vs more extensive surgery (60–62). There is no clear indication for preventive colorectal surgery in *path_PMS2* carriers given the relatively low penetrance of *path_PMS2* variants (56). This means that there is no reason for priority counselling and testing in this case. Decisions regarding the specific operating procedure in this case should be made strictly on patient and tumour characteristics. This has also been included in recent international guidelines (56). The presence or absence of a germline *path_PMS2* variant is not a factor herein.

Case 5

A 65-year-old woman presents with an MSI CRC and isolated loss of PMS2 expression on IHC. No germline *path_MMR* variant is found, nor is there *MLH1* promoter hypermethylation. There is no family history of CRC or EC. The tumour is sent to the pathology department for next generation sequencing of *PMS2*, where one somatic hit in *PMS2* with a variant allele frequency of 23% is

found. There are no signs of loss of heterozygosity indicating loss of the second allele. What would the advice for relatives be in this case?

Case discussion: Per definition, the cause of the dMMR in this case was not found. In the past this would mean that first-degree relatives should be offered a LS-like surveillance scheme. We suggest using new techniques to look for a missed germline variant, such as ultra-long-read sequencing to look for deep intronic variants (or exon deletions), which were recently described as an explanation for part of the missing heritability in up to almost 20% of LS-like cases (63). If such analyses also fail to reveal a germline variant, we would not advise additional screening of the colon or endometrium for relatives as the explanation of the dMMR would most likely be somatic. Naturally, relatives would be encouraged to participate in population-based screening. Of note, the possibility of a (missed) germline mosaicism cannot be excluded, but we believe the chance of vertical transmission to offspring can be considered negligible in this case.

Future

Sequencing strategies

As mentioned in discussion of Case 5, new methods of MMR gene analyses are now available. Ultra-long-read sequencing with reads up to 100 kb could increase the proportion of LS families identified through more efficient detection of both larger deletions and noncoding, deep intronic variants (63–65). The introduction of these strategies is very relevant for *PMS2* because it makes circumvention of pseudogenes much more straightforward (66).

Prevention

Colonoscopic techniques

There has been a debate about whether standard colonoscopy techniques are adequate to detect all colonic lesions in LS. A previous study in France indicated that optimisation of colonoscopy, such as performing chromoendoscopy, and the adjustment of surveillance intervals led to a significant reduction of CRC incidence (67). According to a recent randomised study, the use of white light endoscopy might aid detection of flat lesions, showing a higher detection rate compared to standard colonoscopy (65% vs. 37% respectively, p 0.003) but detecting comparable numbers of total adenomas and right-sided lesions (68). Unfortunately, flat adenomas, which are presumed to harbour more advanced histology, were still frequently missed by current standard colonoscopy. Given the differences in occurrence of benign precursor stages – MMR-DCF for *path_MLH1*, dMMR adenomas for *path_MSH2* and pMMR adenomas for *path_PMS2* carriers (43, 44) – optimisation of colonoscopy procedures would need to be MMR gene–specific.

Chemoprevention

Results from the CAPP2 trial concluded that aspirin intake might prevent nearly half of the CRC diagnosed among individuals affected by LS at low cost and relatively low risk (69). However, there seemed to be a delay in the effect, which only started to be measurable 3–4 years after commencement of chemoprevention, and the effect seemingly occurred when aspirin was taken for at least 2 years. At present the precise mechanism of aspirin in cancer reduction is not known. One hypothesis is modulation of the immune response that potentially enhances T cell activity while suppressing inflammatory responses. An alternative explanation from cell line and mouse model data suggests that aspirin has a pro-apoptotic influence on premalignant cells in the gut, with one conceivable target being MMR-DCF (70). If true, aspirin might have a lower efficacy in *path_PMS2* carriers as they most likely do not develop CRC from MMR-DCF. However, without MMR gene–stratified analyses, this remains speculation.

Vaccination strategies

In the past few years, increased knowledge about immunotherapeutic approaches has also offered additional cancer-preventive strategies for individuals affected by LS. Recent studies revealed that FSP-specific immune responses were already detectable in tumour-free *path_MMR* carriers or individuals with early-stage adenomas, indicating that continuous immunoediting occurs in early LS lesions (71). FSPs derived from cMS variants in dMMR cells can be processed and presented to the host's immune system, potentially triggering immune responses specifically targeting dMMR cells. There is evidence that a high mutational and neoantigen load in tumour cells is associated with the strength of antitumoral immune responses. FSP-based preventive vaccination is currently under investigation, and the first phase I/IIA trial has already demonstrated its safety and immunological effectiveness. The therapy regimen consisted of three FSP neoantigens administered subcutaneously using the adjuvant Montanide ISA 51 in three treatment cycles consisting of four weekly applications. Patients demonstrated considerable response to the FSP neoantigens, and the safety profile was deemed tolerable (72). Investigations of cancer-preventive effects of the FSP vaccine in LS carriers remain to be planned, but recent results from a LS mouse model demonstrated that FSP vaccines can reduce tumour burden and improve survival (73). The presence of a similar cMSI spectrum, and thus of FSPs for dPMS2 tumours (as compared to dMLH1 or dMSH2 tumours), could be reassuring for the expected efficacy of FSP-based vaccines (50). However, the effect of lower immune infiltration remains to be seen. The presence of pMMR adenomas rather than MMR-DCF as a benign precursor in *path_PMS2* carriers could mean that FSPs are presented at a later stage, potentially resulting in a lower efficacy for this type of vaccine. This underlines the need for MMR gene–stratified studies of tumorigenesis in LS.

Immunotherapy

Upregulation of PD-L1 is presumed to be the main immune-evasion mechanism observed in LS-CRC (74, 75). PD-L1 expression has been found in excess in the immune cells at the invasive margin of the tumour bulk as well as the peri- and intra-tumoral macrophages, while its expression on tumour cells was relatively low (76). Multiple studies have now shown the effectiveness of immunotherapy that targets immune checkpoints in dMMR CRC (77–79). PMS2-deficient CRC is unique since it is associated with fewer immune features, such as less pronounced CD3+ T cell infiltration in the tumour milieu (48,

51). This finding indicates that these tumours might develop a lesser degree of immune activation, leading to more aggressive tumour behaviour. It is plausible that dPMS2 tumours use strategies typical of pMMR CRCs to avoid the immune system. However, whether these findings impact the effectiveness of immunotherapy of dPMS2-CRC is still debatable because immune profiling studies that focus on dPMS2-CRC remain scarce. This is an area that needs to be explored further as it could have significant impact on the clinical management of these patients.

Risk-modifying factors

Microbiome

The human intestine is colonised by various types of resident microorganisms, including bacteria, archaea, viruses and fungi, which make up the diverse human microbiota. The impact of dysbiosis in carcinogenesis in the LS population has just begun to be explored. One study has demonstrated a different stool microbiota composition between healthy carriers of LS and LS-CRC patients (80). Another study concluded that stool samples of LS patients with adenoma showed lower amounts of *Clostridiaceae* and increased amounts of *Lachnospiraceae, Desulfovibrio* sp. and *Ruminococcaceae*. Interestingly, this study also revealed that the underlying germline *path_MMR* variant also affected the microbial species observed. A decreased amount of *Blautia* sp. was observed in *path_MLH1* and *path_MSH2* carriers, while an enriched abundance of *C. bartletti* and *Alistipes* sp. was observed in samples from *path_PMS2* carriers (81). From the results described above, it is evident that our understanding of the reciprocal association between gut dysbiosis and LS-CRC development is far from complete. Complex *in vitro* culturing models such as organoids and organ-on-a-chip will aid in answering these questions, including a delineation of cause versus consequence. It is also conceivable that different microorganisms play a role at different stages of tumour evolution, which is why it is also extremely relevant to take the different pathways of CRC development into account. For example, the microbiome could have an important influence on the development of pMMR adenomas, which are most likely the benign precursors for CRCs in *path_PMS2* carriers, as described above. The gut microbiome therefore has potential to be a controllable factor in *path_PMS2* carriers.

Lifestyle factors

Lifestyle factors such as smoking habits, physical activity and BMI have been associated with modification of CRC risk among *path_MMR* carriers (82, 83). Previous studies have firmly established the correlation between dietary intake of certain nutrients and increased risk of intestinal inflammation (84, 85). The GeoLynch study conducted in the Netherlands further confirmed that diets rich in processed meat, sugar and refined grains were thought to support the inflammatory process in the gut, which might eventually promote carcinogenesis in LS-CRC (86). In contrast, diets rich in fibre, vegetables, legumes and fish were associated with a reduced risk of sporadic CRC formation. Recently, a study on the preventive use of resistant starch in LS patients showed no impact on CRC risk but did find a significant reduction in extra-colonic cancer (87, 88). Since most patients were *path_MLH1* (60%) or *path_MSH2* carriers (37%), with only a few *path_MSH6* (3%) and no *path_PMS2* carriers, the effect of the use of resistant starch in these two last groups remains a question. Previously studied cohorts were also underpowered to look at MMR gene–specific effects of lifestyle factors. Whether improving the lifestyle of LS carriers would confer a later age of CRC onset, milder type of disease, or no CRC manifestation at all also still needs to be addressed.

While results are somewhat conflicting, most studies find no differences in risk profiles including BMI, co-morbidity and lifestyle factors between sporadic and LS-EC (89). For example, higher BMI seems to increase EC risk in both groups. In contrast, some hormonal factors appear to have a risk-lowering effect, although here again there was no difference between sporadic and LS-EC (90). This suggests that these factors might not have MMR gene–specific effects, but future research should shed more light on this.

Digenic inheritance of pathogenic variants in other genes

The reduced penetrance of a pathogenic variant might be caused by either genetic or non-genetic factors, collectively influencing disease manifestation. Presence of disease-modifying variants or the occurrence of different somatic variants might explain the varying penetrance of the germline variant, since less common germline variants in the human genome may modify the expression of major genes. Digenic inheritance is the phenomenon in which presence of genetic variants (inherited or arising *de novo*) impacts both the penetrance of the gene of interest and the observed phenotype. It further emphasises the complex genotype–phenotype interactions (91). Digenic inheritance is considered a major contributor to the variable phenotypes observed in hereditary disorders, which might modify predisposition to illness or cause heterogeneity in disease manifestation among family members. Digenic inheritance has also been described in Lynch syndrome (92). The facts that *path_PMS2* carriers do not develop cancer as frequently as other *path_MMR* carriers and members of the same family affected by the same *path_PMS2* variant also demonstrate a wide range of age for cancer-onset offer potential evidence of digenic inheritance among this group of carriers. Indeed, a very recent case-report described two teenage siblings with multiple adenomas and CRC with a maternally inherited *path_PMS2* variant and a paternally inherited *path_POLD1* variant (93). Interestingly, molecular studies of the tumours revealed an ultra-mutated tumour phenotype with mutational signatures of both *PMS2* and *POLD1*, suggesting that these factors interacted to cause the relatively severe clinical phenotype in these cases.

Efforts to discover other potential genetic modifiers or epigenetic events that could contribute to LS manifestations, especially among *path_PMS2* carriers, have to be pursued as they have evident consequences for clinical management.

Influence of HLA genotype

As described above, development of CRC is influenced by activation of the immune system. The presence of immunogenic FSPs has the potential to activate the immune system and thereby

prevent early dMMR lesions from progressing to CRC. A key factor in the presentation of FSPs to immune surveillance is the HLA complex. This has led to the hypothesis that HLA genotype could be an explanation for the observed differences in phenotype, i.e. differences in cancer risks and age of onset. No data on this is currently available, but a new initiative has been established to further investigate this (INDICATE, http://indicate-lynch.org/) (94). Analysing such data in a MMR gene–stratified manner seems critical given the differences in preferential carcinogenic pathways. Hypothetically, the presence of pMMR adenomas as the benign precursor of CRC in *path_PMS2* carriers could mean that the HLA genotype is not as strong a predictor for cancer risk in these carriers because of the absence of FSPs in early lesions, which could be targeted by early immune surveillance.

Conclusion

In the past decade it has become apparent that clinical and biological characteristics of LS patients are highly dependent on the specific MMR gene affected. PMS2-LS clearly represents the milder end of the phenotypic spectrum and has its own unique pathophysiology. Surveillance guidelines now recommend a starting age of 35 years for colonoscopy for *path_PMS2* carriers and take a (very) conservative approach towards gynaecological surveillance and, more specifically, prophylactic surgery. While the cancer risks are relatively low, as dPMS2 most likely occurs later in tumour development, these tumours share characteristics with dMMR CRC as well as sporadic pMMR CRC. This could result in clinically important differences with regard to (chemo)prevention and therapy. The improvement of fundamental research techniques and increased detection of *path_PMS2* carriers will lead to a more thorough understanding of this specific subset of LS patients and aid in clinical decision-making in the future.

Author contributions

KA, MN, KK, RS and SB contributed to conception and design of the review. KA and SB wrote the first draft of the review. All authors contributed to the article and approved the submitted version.

Funding

KA and SB are being funded by the Dutch Cancer Society. Grant number: KWF 2019-2/12570.

Acknowledgments

Assistance with writing by Kate McIntyre is gratefully acknowledged. Parts of this review have previously been published online in the thesis of SB (PMS2-associated Lynch Syndrome: the odd one out, Leiden University repository, 2018).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Win AK, Jenkins MA, Dowty JG, Antoniou AC, Lee A, Giles GG, et al. Prevalence and penetrance of major genes and polygenes for colorectal cancer. *Cancer Epidemiol Biomarkers Prev* (2017) 26(3):404–12. doi: 10.1158/1055-9965.EPI-16-0693

2. Senter L, Clendenning M, Sotamaa K, Hampel H, Green J, Potter JD, et al. The clinical phenotype of lynch syndrome due to germ-line PMS2 mutations. *Gastroenterology* (2008) 135(2):419–28. doi: 10.1053/j.gastro.2008.04.026

3. Ten Broeke SW, van der Klift HM, Tops CMJ, Aretz S, Bernstein I, Buchanan DD, et al. Cancer risks for PMS2-associated lynch syndrome. *J Clin Oncol* (2018) 36(29):2961–8. doi: 10.1200/JCO.2018.78.4777

4. ten Broeke SW, Brohet RM, Tops CM, van der Klift HM, Velthuizen ME, Bernstein I, et al. Lynch syndrome caused by germline PMS2 mutations: delineating the cancer risk. *J Clin Oncol* (2015) 33(4):319–25. doi: 10.1200/JCO.2014.57.8088

5. Peltomaki P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. *J Clin Oncol* (2003) 21(6):1174–9. doi: 10.1200/JCO.2003.04.060

6. Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* (1994) 371(6492):75–80. doi: 10.1038/371075a0

7. van der Klift HM, Tops CM, Bik EC, Boogaard MW, Borgstein AM, Hansson KB, et al. Quantification of sequence exchange events between PMS2 and PMS2CL provides a basis for improved mutation scanning of lynch syndrome patients. *Hum Mutat* (2010) 31 (5):578–87. doi: 10.1002/humu.21229

8. van der Klift HM, Mensenkamp AR, Drost M, Bik EC, Vos YJ, Gille HJ, et al. Comprehensive mutation analysis of PMS2 in a large cohort of probands suspected of lynch syndrome or constitutional mismatch repair deficiency syndrome. *Hum Mutat* (2016) 37(11):1162–79. doi: 10.1002/humu.23052

9. Clendenning M, Hampel H, LaJeunesse J, Lindblom A, Lockman J, Nilbert M, et al. Long-range PCR facilitates the identification of PMS2-specific mutations. *Hum Mutat* (2006) 27(5):490–5. doi: 10.1002/humu.20318. Erratum in: Hum Mutat

10. Vasen HF, Möslein G, Alonso A, Aretz S, Bernstein I, Bertario L, et al. Recommendations to improve identification of hereditary and familial colorectal cancer in europe. *Fam Cancer.* (2010) 9(2):109–15. doi: 10.1007/s10689-009-9291-3

11. Clendenning M, Senter L, Hampel H, Robinson KL, Sun S, Buchanan D, et al. A frame-shift mutation of PMS2 is a widespread cause of lynch syndrome. *J Med Genet* (2008) 45(6):340–5. doi: 10.1136/jmg.2007.056150

12. Truninger K, Menigatti M, Luz J, Russell A, Haider R, Gebbers JO, et al. Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. *Gastroenterology* (2005) 128(5):1160–71. doi: 10.1053/j.gastro.2005.01.056

13. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Feasibility of screening for lynch syndrome among patients with colorectal cancer. *J Clin Oncol* (2008) 26(35):5783–8. doi: 10.1200/JCO.2008.17.5950

14. Baudhuin LM, Burgart LJ, Leontovich O, Thibodeau SN. Use of microsatellite instability and immunohistochemistry testing for the identification of individuals at risk for lynch syndrome. *Fam Cancer.* (2005) 4(3):255–65. doi: 10.1007/s10689-004-1447-6

15. Halvarsson B, Lindblom A, Rambech E, Lagerstedt K, Nilbert M. The added value of PMS2 immunostaining in the diagnosis of hereditary nonpolyposis colorectal cancer. *Fam Cancer.* (2006) 5(4):353–8. doi: 10.1007/s10689-006-0005-9

16. Eikenboom EL, van der Werf-'t Lam AS, Rodríguez-Girondo M, Van Asperen CJ, Dinjens WNM, Hofstra RMW, et al. Universal immunohistochemistry for lynch syndrome: A systematic review and meta-analysis of 58,580 colorectal carcinomas. *Clin Gastroenterol Hepatol* (2022) 20(3):e496–507. doi: 10.1016/j.cgh.2021.04.021

17. Pearlman R, Haraldsdottir S, de la Chapelle A, Jonasson JG, Liyanarachchi S, Frankel WL, et al. Clinical characteristics of patients with colorectal cancer with double somatic mismatch repair mutations compared with lynch syndrome. *J Med Genet* (2019) 56(7):462–70. doi: 10.1136/jmedgenet-2018-105698

18. Haraldsdottir S, Rafnar T, Frankel WL, Einarsdottir S, Sigurdsson A, Hampel H, et al. Comprehensive population-wide analysis of lynch syndrome in iceland reveals founder mutations in MSH6 and PMS2. *Nat Commun* (2017) 8:14755. doi: 10.1038/ncomms14755

19. Herkert JC, Niessen RC, Olderode-Berends MJ, Veenstra-Knol HE, Vos YJ, van der Klift HM, et al. Paediatric intestinal cancer and polyposis due to bi-allelic PMS2 mutations: case series, review and follow-up guidelines. *Eur J Cancer.* (2011) 47(7):965–82. doi: 10.1016/j.ejca.2011.01.013

20. Adar T, Rodgers LH, Shannon KM, Yoshida M, Ma T, Mattia A, et al. Universal screening of both endometrial and colon cancers increases the detection of lynch syndrome. *Cancer* (2018) 124(15):3145–53. doi: 10.1002/cncr.31534

21. Ryan E, Sheahan K, Creavin B, Mohan HM, Winter DC. The current value of determining the mismatch repair status of colorectal cancer: A rationale for routine testing. *Crit Rev Oncol Hematol* (2017) 116:38–57. doi: 10.1016/j.critrevonc.2017.05.006

22. van Lier MG, Leenen CH, Wagner A, Ramsoekh D, Dubbink HJ, van den Ouweland AM, et al. Yield of routine molecular analyses in colorectal cancer patients ≤70 years to detect underlying lynch syndrome. *J Pathol* (2012) 226(5):764–74. doi: 10.1002/path.3963

23. Møller P, Seppälä T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Cancer incidence and survival in lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective lynch syndrome database. *Gut* (2017) 66(3):464–72. doi: 10.1136/gutjnl-2015-309675

24. Vos JR, Hsu L, Brohet RM, Mourits MJ, de Vries J, Malone KE, et al. Bias correction methods explain much of the variation seen in breast cancer risks of BRCA1/2 mutation carriers. *J Clin Oncol* (2015) 33(23):2553–62. doi: 10.1200/JCO.2014.59.0463

25. Jackson L, W. M, Harrison JW, Wood AR, Ruth KS, Tyrrell J, et al. Influence of family history on penetrance of hereditary cancers in a population setting. *MedRxiv* (2022). doi: 10.1101/2022.07.08.22277415

26. Bougeard G, Olivier-Faivre L, Baert-Desurmont S, Tinat J, Martin C, Bouvignies E, et al. Diversity of the clinical presentation of the MMR gene biallelic mutations. *Fam Cancer.* (2014) 13(1):131–5. doi: 10.1007/s10689-013-9676-1

27. Shah KN. The diagnostic and clinical significance of cafe-au-lait macules. *Pediatr Clin North Am* (2010) 57(5):1131–53. doi: 10.1016/j.pcl.2010.07.002

28. Suerink M, Potjer TP, Versluijs AB, Ten Broeke SW, Tops CM, Wimmer K, et al. Constitutional mismatch repair deficiency in a healthy child: On the spot diagnosis? *Clin Genet* (2018) 93(1):134–7. doi: 10.1111/cge.13053

29. Wimmer K, Kratz CP, Vasen HF, Caron O, Colas C, Entz-Werle N, et al. Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the european consortium 'care for CMMRD' (C4CMMRD). *J Med Genet* (2014) 51(6):355–65. doi: 10.1136/jmedgenet-2014-102284

30. Suerink M, Rodríguez-Girondo M, van der Klift HM, Colas C, Brugieres L, Lavoine N, et al. An alternative approach to establishing unbiased colorectal cancer risk estimation in lynch syndrome. *Genet Med* (2019) 21(12):2706–12. doi: 10.1038/s41436-019-0577-z

31. Dominguez-Valentin M, Sampson JR, Seppälä TT, Ten Broeke SW, Plazzer JP, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the prospective lynch syndrome database. *Genet Med* (2020) 22(1):15–25. doi: 10.1038/s41436-019-0596-9

32. Møller P, Seppälä T, Dowty JG, Haupt S, Dominguez-Valentin M, Sunde L, et al. Colorectal cancer incidences in lynch syndrome: a comparison of results from the prospective lynch syndrome database and the international mismatch repair consortium. *Hered Cancer Clin Pract* (2022) 20(1):36. doi: 10.1186/s13053-022-00241-1

33. Møller P, Seppälä TT, Bernstein I, Holinski-Feder E, Sala P, Gareth Evans D, et al. Cancer risk and survival in *path_MMR* carriers by gene and gender up to 75 years of age: a report from the prospective lynch syndrome database. *Gut* (2018) 67(7):1306–16. doi: 10.1136/gutjnl-2017-314057

34. International Mismatch Repair, C. Variation in the risk of colorectal cancer in families with lynch syndrome: a retrospective cohort study. *Lancet Oncol* (2021) 22(7):1014–22. doi: 10.1016/S1470-2045(21)00189-3

35. Dominguez-Valentin M, Plazzer JP, Sampson JR, Engel C, Aretz S, Jenkins MA, et al. No difference in penetrance between truncating and Missense/Aberrant splicing pathogenic variants in *MLH1* and *MSH2*: A prospective lynch syndrome database study. *J Clin Med* (2021) 10(13):2856. doi: 10.3390/jcm10132856

36. Peltomaki P. Update on lynch syndrome genomics. *Fam Cancer* (2016) 15(3):385–93. doi: 10.1007/s10689-016-9882-8

37. Suerink M, van der Klift HM, Ten Broeke SW, Dekkers OM, Bernstein I, Capellá Munar G, et al. The effect of genotypes and parent of origin on cancer risk and age of cancer development in PMS2 mutation carriers. *Genet Med* (2016) 18(4):405–9. doi: 10.1038/gim.2015.83

38. Li L, Hamel N, Baker K, McGuffin MJ, Couillard M, Gologan A, et al. A homozygous PMS2 founder mutation with an attenuated constitutional mismatch repair deficiency phenotype. *J Med Genet* (2015) 52(5):348–52. doi: 10.1136/jmedgenet-2014-102934

39. Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol* (2006) 7(5):335–46. doi: 10.1038/nrm1907

40. Jahid S, Lipkin S. Mouse models of inherited cancer syndromes. *Hematol Oncol Clin North Am* (2010) 24(6):1205–28. doi: 10.1016/j.hoc.2010.08.011

41. Knudson AGJr. Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl. Acad. Sci. U S A* (1971) 68(4):820–3. doi: 10.1073/pnas.68.4.820

42. Umar A. Lynch syndrome (HNPCC) and microsatellite instability. *Dis Markers* (2004) 20(4-5):179–80. doi: 10.1155/2004/486032

43. Ahadova A, Gallon R, Gebert J, Ballhausen A, Endris V, Kirchner M, et al. Three molecular pathways model colorectal carcinogenesis in lynch syndrome. *Int J Cancer.* (2018) 143(1):139–50. doi: 10.1002/ijc.31300

44. Engel C, Ahadova A, Seppälä TT, Aretz S, Bigirwamungu-Bargeman M, Bläker H, et al. Associations of pathogenic variants in MLH1, MSH2, and MSH6 with risk of colorectal adenomas and tumors and with somatic mutations in patients with lynch syndrome. *Gastroenterology* (2020) 158(5):1326–33. doi: 10.1053/j.gastro.2019.12.032

45. Kloor M, Huth C, Voigt AY, Benner A, Schirmacher P, von Knebel Doeberitz M, et al. Prevalence of mismatch repair-deficient crypt foci in lynch syndrome: a pathological study. *Lancet Oncol* (2012) 13(6):598–606. doi: 10.1016/S1470-2045(12)70109-2

46. Staffa L, Echterdiek F, Nelius N, Benner A, Werft W, Lahrmann B, et al. Mismatch repair-deficient crypt foci in lynch syndrome–molecular alterations and association with clinical parameters. *PloS One* (2015) 10(3):e0121980. doi: 10.1371/journal.pone.0121980

47. Ahadova A, von Knebel Doeberitz M, Bläker H, Kloor M. CTNNB1-mutant colorectal carcinomas with immediate invasive growth: a model of interval cancers in lynch syndrome. *Fam Cancer.* (2016) 15(4):579–86. doi: 10.1007/s10689-016-9899-z

48. Ten Broeke SW, van Bavel TC, Jansen AML, Gómez-García E, Hes FJ, van Hest LP, et al. Molecular background of colorectal tumors from patients with lynch syndrome associated with germline variants in PMS2. *Gastroenterology* (2018) 155(3):844–51. doi: 10.1053/j.gastro.2018.05.020

49. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. *Nature* (2013) 500(7463):415–21. doi: 10.1038/nature12477

50. Bajwa-Ten Broeke SW, Ballhausen A, Ahadova A, Suerink M, Bohaumilitzky L, Seidler F, et al. The coding microsatellite mutation profile of PMS2-deficient colorectal cancer. *Exp Mol Pathol* (2021) 122:104668. doi: 10.1016/j.yexmp.2021.104668

51. Alpert L, Pai RK, Srivastava A, McKinnon W, Wilcox R, Yantiss RK, et al. Colorectal carcinomas with isolated loss of PMS2 staining by immunohistochemistry. *Arch Pathol Lab Med* (2018) 142(4):523–8. doi: 10.5858/arpa.2017-0156-OA

52. Wong S, Hui P, Buza N. Frequent loss of mutation-specific mismatch repair protein expression in nonneoplastic endometrium of lynch syndrome patients. *Mod Pathol* (2020) 33(6):1172–81. doi: 10.1038/s41379-020-0455-x

53. Crosbie EJ, Ryan NAJ, Arends MJ, Bosse T, Burn J, Cornes JM, et al. The manchester international consensus group recommendations for the management of gynecological cancers in lynch syndrome. *Genet Med* (2019) 21(10):2390–400. doi: 10.1038/s41436-019-0489-y

54. van Leerdam ME, Roos VH, van Hooft JE, Balaguer F, Dekker E, Kaminski MF, et al. Endoscopic management of lynch syndrome and of familial risk of colorectal cancer: European society of gastrointestinal endoscopy (ESGE) guideline. *Endoscopy* (2019) 51(11):1082–93. doi: 10.1055/a-1016-4977

55. Monahan KJ, Bradshaw N, Dolwani S, Desouza B, Dunlop MG, East JE, et al. Guidelines for the management of hereditary colorectal cancer from the british society of gastroenterology (BSG)/Association of coloproctology of great britain and ireland (ACPGBI)/United kingdom cancer genetics group (UKCGG). *Gut* (2020) 69(3):411–44. doi: 10.1136/gutjnl-2019-319915

56. Seppälä TT, Latchford A, Negoi I, Sampaio Soares A, Jimenez-Rodriguez R, Sánchez-Guillén L, et al. European guidelines from the EHTG and ESCP for lynch syndrome: an updated third edition of the mallorca guidelines based on gene and gender. *Br J Surg* (2021) 108(5):484–98. doi: 10.1002/bjs.11902

57. Kang YJ, Caruana M, McLoughlin K, Killen J, Simms K, Taylor N, et al. The predicted effect and cost-effectiveness of tailoring colonoscopic surveillance according to mismatch repair gene in patients with lynch syndrome. *Genet Med* (2022) 24(9):1831–46. doi: 10.1016/j.gim.2022.05.016

58. Kastrinos F, Ingram MA, Silver ER, Oh A, Laszkowska M, Rustgi AK, et al. Gene-specific variation in colorectal cancer surveillance strategies for lynch syndrome. *Gastroenterology* (2021) 161(2):453–462.e15. doi: 10.1053/j.gastro.2021.04.010

59. Miller DT, Lee K, Abul-Husn NS, Amendola LM, Brothers K, Chung WK, et al. ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the american college of medical genetics and genomics (ACMG). *Genet Med* (2022) 24(7):1407–14. doi: 10.1016/j.gim.2022.04.006

60. Heneghan HM, Martin ST, Winter DC. Segmental vs extended colectomy in the management of hereditary nonpolyposis colorectal cancer: a systematic review and meta-analysis. *Colorectal Dis* (2015) 17(5):382–9. doi: 10.1111/codi.12868

61. Anele CC, Adegbola SO, Askari A, Rajendran A, Clark SK, Latchford A, et al. Risk of metachronous colorectal cancer following colectomy in lynch syndrome: a systematic review and meta-analysis. *Colorectal Dis* (2017) 19(6):528–36. doi: 10.1111/codi.13679

62. Malik SS, Lythgoe MP, McPhail M, Monahan KJ. Metachronous colorectal cancer following segmental or extended colectomy in lynch syndrome: a systematic review and meta-analysis. *Fam Cancer.* (2018) 17(4):557–64. doi: 10.1007/s10689-017-0062-2

63. Te Paske IBAW, Mensenkamp AR, Neveling KERN-GENTURIS Lynch-Like Working Group, , Hoogerbrugge N, Ligtenberg MJL, et al. Noncoding aberrations in

mismatch repair genes underlie a substantial part of the missing heritability in lynch syndrome. *Gastroenterology* (2022) 163(6):1691–1694.e7. doi: 10.1053/j.gastro.2022.08.041

64. Kono N, Arakawa K. Nanopore sequencing: Review of potential applications in functional genomics. *Dev Growth Differ* (2019) 61(5):316–26. doi: 10.1111/dgd.12608

65. Jain M, Koren S, Miga KH, Quick J, Rand AC, Sasani TA, et al. Nanopore sequencing and assembly of a human genome with ultra-long reads. *Nat Biotechnol* (2018) 36(4):338–45. doi: 10.1038/nbt.4060

66. Leija-Salazar M, Sedlazeck FJ, Toffoli M, Mullin S, Mokretar K, Athanasopoulou M, et al. Evaluation of the detection of GBA missense mutations and other variants using the oxford nanopore MinION. *Mol Genet Genomic Med* (2019) 7(3):e564. doi: 10.1002/mgg3.564

67. Perrod G, Samaha E, Rahmi G, Khater S, Abbes L, Savale C, et al. Impact of an optimized colonoscopic screening program for patients with lynch syndrome: 6-year results of a specialized french network. *Therap Adv Gastroenterol* (2018) 11:1756284818775058. doi: 10.1177/1756284818775058

68. Rivero-Sánchez L, Arnau-Collell C, Herrero J, Remedios D, Cubiella J, García-Cougil M, et al. White-light endoscopy is adequate for lynch syndrome surveillance in a randomized and noninferiority study. *Gastroenterology* (2020) 158(4):895–904.e1. doi: 10.1053/j.gastro.2019.09.003

69. Burn J, Mathers J, Bishop DT. Lynch syndrome: history, causes, diagnosis, treatment and prevention (CAPP2 trial). *Dig Dis* (2012) 30 Suppl 2:39–47. doi: 10.1159/000341892

70. Burn J, Sheth H, Elliott F, Reed L, Macrae F, Mecklin JP, 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(10240):1855–63. doi: 10.1016/S0140-6736(20)30366-4

71. Schwitalle Y, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, et al. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. *Gastroenterology* (2008) 134(4):988–97. doi: 10.1053/j.gastro.2008.01.015

72. Kloor M, Reuschenbach M, Pauligk C, Karbach J, Rafiyan MR, Al-Batran SE, et al. A frameshift peptide neoantigen-based vaccine for mismatch repair-deficient cancers: A phase I/IIa clinical trial. *Clin Cancer Res* (2020) 26(17):4503–10. doi: 10.1158/1078-0432.CCR-19-3517

73. Gebert J, Gelincik O, Oezcan-Wahlbrink M, Marshall JD, Hernandez-Sanchez A, Urban K, et al. Recurrent frameshift neoantigen vaccine elicits protective immunity with reduced tumor burden and improved overall survival in a lynch syndrome mouse model. *Gastroenterology* (2021) 161(4):1288–1302.e13. doi: 10.1053/j.gastro.2021.06.073

74. Dudley JC, Lin MT, Le DT, Eshleman JR. Microsatellite instability as a biomarker for PD-1 blockade. *Clin Cancer Res* (2016) 22(4):813–20. doi: 10.1158/1078-0432.CCR-15-1678

75. Gelsomino F, Barbolini M, Spallanzani A, Pugliese G, Cascinu S. The evolving role of microsatellite instability in colorectal cancer: A review. *Cancer Treat Rev* (2016) 51:19–26. doi: 10.1016/j.ctrv.2016.10.005

76. Liu X, He S, Wu H, Xie H, Zhang T, Deng Z. Blocking the PD-1/PD-L1 axis enhanced cisplatin chemotherapy in osteosarcoma in vitro and *in vivo*. *Environ Health Prev Med* (2019) 24(1):79. doi: 10.1186/s12199-019-0835-3

77. Le DT, Kim TW, Van Cutsem E, Geva R, Jäger D, Hara H, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-High/Mismatch repair-deficient metastatic colorectal cancer: KEYNOTE-164. *J Clin Oncol* (2020) 38(1):11–9. doi: 10.1200/JCO.19.02107

78. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite Instability/Mismatch repair-deficient cancer: Results from the phase II KEYNOTE-158 study. *J Clin Oncol* (2020) 38(1):1–10. doi: 10.1200/JCO.19.02105

79. O'Malley DM, Bariani GM, Cassier PA, Marabelle A, Hansen AR, De Jesus Acosta A, et al. Pembrolizumab in patients with microsatellite instability-high advanced endometrial cancer: Results from the KEYNOTE-158 study. *J Clin Oncol* (2022) 40(7):752–61. doi: 10.1200/JCO.21.01874

80. Gonzalez A, Kapila N, Melendez-Rosado J, Liang H, Castro-Pavia F. An evaluation of the fecal microbiome in lynch syndrome. *J Gastrointest Cancer*. (2021) 52(1):365–8. doi: 10.1007/s12029-021-00588-z

81. Yan Y, Drew DA, Markowitz A, Lloyd-Price J, Abu-Ali G, Nguyen LH, et al. Structure of the mucosal and stool microbiome in lynch syndrome. *Cell Host Microbe* (2020) 27(4):585–600.e4. doi: 10.1016/j.chom.2020.03.005

82. van Duijnhoven FJ, Botma A, Winkels R, Nagengast FM, Vasen HF, Kampman E. Do lifestyle factors influence colorectal cancer risk in lynch syndrome? *Fam Cancer* (2013) 12(2):285–93. doi: 10.1007/s10689-013-9645-8

83. Dashti SG, Win AK, Hardikar SS, Glombicki SE, Mallenahalli S, Thirumurthi S, et al. Physical activity and the risk of colorectal cancer in lynch syndrome. *Int J Cancer*. (2018) 143(9):2250–60. doi: 10.1002/ijc.31611

84. Day SD, Enos RT, McClellan JL, Steiner JL, Velázquez KT, Murphy EA. Linking inflammation to tumorigenesis in a mouse model of high-fat-diet-enhanced colon cancer. *Cytokine* (2013) 64(1):454–62. doi: 10.1016/j.cyto.2013.04.031

85. Brouwer JG, Makama M, van Woudenbergh GJ, Vasen HF, Nagengast FM, Kleibeuker JH, et al. Inflammatory potential of the diet and colorectal tumor risk in persons with lynch syndrome. *Am J Clin Nutr* (2017) 106(5):1287–94. doi: 10.3945/ajcn.117.152900

86. Botma A, Vasen HF, van Duijnhoven FJ, Kleibeuker JH, Nagengast FM, Kampman E. Dietary patterns and colorectal adenomas in lynch syndrome: the GEOLynch cohort study. *Cancer* (2013) 119(3):512–21. doi: 10.1002/cncr.27726

87. Mathers JC, Elliott F, Macrae F, Mecklin JP, Möslein G, McRonald FE, et al. Cancer prevention with resistant starch in lynch syndrome patients in the CAPP2-randomized placebo controlled trial: Planned 10-year follow-up. *Cancer Prev Res (Phila)*. (2022) 15(9):623–34. doi: 10.1158/1940-6207.CAPR-22-0044

88. Mathers JC, Movahedi M, Macrae F, Mecklin JP, Moeslein G, Olschwang S, et al. Long-term effect of resistant starch on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. *Lancet Oncol* (2012) 13(12):1242–9. doi: 10.1016/S1470-2045(12)70475-8

89. Bounous VE, Robba E, Perotto S, Pasini B, Tomasi Cont N, Ricci MT, et al. Gynecological cancers in lynch syndrome: A comparison of the histological features with sporadic cases of the general population. *J Clin Med* (2022) 11(13):3689. doi: 10.3390/jcm11133689

90. Dashti SG, Chau R, Ouakrim DA, Buchanan DD, Clendenning M, Young JP, et al. Female hormonal factors and the risk of endometrial cancer in lynch syndrome. *JAMA* (2015) 314(1):61–71. doi: 10.1001/jama.2015.6789

91. Deltas C. Digenic inheritance and genetic modifiers. *Clin Genet* (2018) 93(3):429–38. doi: 10.1111/cge.13150

92. Vogelaar IP, Greer S, Wang F, Shin G, Lau B, Hu Y, et al. Large cancer pedigree involving multiple cancer genes including likely digenic *MSH2* and *MSH6* lynch syndrome (LS) and an instance of recombinational rescue from LS. *Cancers (Basel)*. (2022) 15(1):228. doi: 10.3390/cancers15010228

93. Schamschula E, Kinzel M, Wernstedt A, Oberhuber K, Gottschling H, Schnaiter S, et al. Teenage-onset colorectal cancers in a digenic cancer predisposition syndrome provide clues for the interaction between mismatch repair and polymerase δ proofreading deficiency in tumorigenesis. *Biomolecules* (2022) 12(10):1350. doi: 10.3390/biom12101350

94. Ahadova A, Witt J, Haupt S, Gallon R, Hüneburg R, Nattermann J, et al. Is HLA type a possible cancer risk modifier in lynch syndrome? *Int J Cancer* (2022). doi: 10.1002/ijc.34312

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 01 March 2023
DOI 10.3389/fonc.2023.1127683

Check for updates

OPEN ACCESS

EDITED BY
Toni T. Seppälä,
Helsinki University Central Hospital, Finland

REVIEWED BY
Annika Auranen,
Tampere University Hospital, Finland
Emma J. Crosbie,
The University of Manchester,
United Kingdom

*CORRESPONDENCE
Kari L. Ring
KEL7J@hscmail.mcc.virginia.edu

SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 19 December 2022
ACCEPTED 16 February 2023
PUBLISHED 01 March 2023

CITATION
Underkofler KA and Ring KL (2023) Updates in gynecologic care for individuals with lynch syndrome.
Front. Oncol. 13:1127683.
doi: 10.3389/fonc.2023.1127683

COPYRIGHT
© 2023 Underkofler and Ring. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Updates in gynecologic care for individuals with lynch syndrome

Kaylee A. Underkofler and Kari L. Ring*

Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University of Virginia, Charlottesville, VA, United States

Lynch syndrome is an autosomal dominant hereditary cancer syndrome caused by germline pathogenic variants (PVs) in DNA mismatch repair genes (*MLH1*, *MSH2*, *PMS2*, *MSH6*) or the *EPCAM* gene. It is estimated to affect 1 in 300 individuals and confers a lifetime risk of cancer of 10-90%, depending on the specific variant and type of cancer. Lynch syndrome is the most common cause of inherited colorectal cancer, but for women, endometrial cancer is more likely to be the sentinel cancer. There is also evidence that certain PVs causing Lynch syndrome confer an increased risk of ovarian cancer, while the risk of ovarian cancer in others is not well defined. Given this, it is essential for the practicing gynecologist and gynecologic oncologist to remain up to date on the latest techniques in identification and diagnosis of individuals with Lynch syndrome as well as evidence-based screening and risk reduction recommendations for those impacted. Furthermore, as the landscape of gynecologic cancer treatment shifts towards treatment based on molecular classification of tumors, knowledge of targeted therapies well-suited for mismatch repair deficient Lynch tumors will be crucial. The objective of this review is to highlight recent updates in the literature regarding identification and management of individuals with Lynch syndrome as it pertains to endometrial and ovarian cancers to allow gynecologic providers the opportunity to both prevent and identify Lynch-associated cancers earlier, thereby reducing the morbidity and mortality of the syndrome.

KEYWORDS

lynch syndrome, endometrial cancer, ovarian cancer, genetics, gynecology, gynecologic oncology

1 Introduction

Lynch syndrome, first recognized in 1895, is a well-defined hereditary cancer syndrome that affects approximately 1 in 300 individuals in the general population (1, 2). It is an autosomal dominant condition that is caused by pathogenic variants (PVs) in DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *PMS2*, *MSH6*) or the *EPCAM* gene, which causes upstream promoter hypermethylation of *MSH2*. The lifetime risk of developing cancer among those with Lynch syndrome is highly variable, ranging from 10-90%, and is now understood to be related to the specific pathogenic variant (PV) causing the disorder in an individual or family (3–5). For example, those with a PV in *MLH1* have a 71-90% lifetime risk of any Lynch cancer and a 35-90% lifetime risk of colorectal cancer whereas

those with a PV in *PMS2* have a 34-52% lifetime risk of any Lynch cancer and a 12-52% risk of colorectal cancer.

While it was first defined for its association with colon cancer, Lynch syndrome also increases the lifetime risk of cancer of the endometrium, ovary, stomach, small bowel, pancreas, brain, and genitourinary system (6). Endometrial cancer is the most common extracolonic cancer and is often the sentinel malignancy in women (7). Lynch syndrome is thought to cause 3% of colon cancers, and it is also thought to cause 3% of endometrial cancers (8–11). Endometrial cancer is most strongly associated with PVs in *MLH1*, *MSH2*, and *MSH6*, which confer a lifetime risk of endometrial cancer of 34-54%, 21-57%, and 16-49%, respectively (3–5, 12). Ovarian cancer is also associated with Lynch syndrome, specifically with PVs in *MSH2* and *MLH1*, which confer an 8-38% and 4-20% lifetime risk of ovarian cancer, respectively. In contrast, updated evidence in *MSH6* and *PMS2* carriers does not show a definitive increased lifetime risk of ovarian cancer, which is different from broader non-variant risk estimates utilized in the past (Table 1). Lynch associated cancers are also diagnosed at an earlier age than their general population counterparts (9). The mean age at the time of endometrial cancer diagnosis in those with Lynch syndrome is 47-55 years compared to age 60 in those without Lynch syndrome, and this same pattern is observed with ovarian cancer (13).

These facts highlight the importance of women's health provider familiarity with Lynch syndrome. Methods of successful endometrial cancer risk reduction among women with Lynch Syndrome have been identified, such as total hysterectomy (14). Therefore, this condition should be in the forefront of the gynecologist's and gynecologic oncologist's mind when seeing patients with endometrial or ovarian cancer or a suggestive personal or family history to assist with preventive efforts. Women's health providers should be prepared to discuss the diagnosis, lifetime risk of malignancy, as well as recommended screening and risk reduction methods. Gynecologic oncologists can take this discussion a step further with recent evidence supporting targeted treatments for MMR deficient tumors associated with Lynch syndrome. The objective of this review is to highlight recent updates in the literature regarding these topics of identification and management of individuals with Lynch syndrome as it pertains to endometrial and ovarian cancers. This may allow gynecologic providers the opportunity to both prevent and identify Lynch-associated cancers earlier, thereby reducing the morbidity and mortality of the syndrome.

2 Identification of individuals with lynch syndrome

The first step in reducing morbidity and mortality of Lynch syndrome on a population level is to identify which individuals may be at risk and qualify for germline genetic testing. This unfortunately is also one of the most challenging steps. Lynch syndrome is suspected to be underdiagnosed in the general population (15). Many screening tools have been created over the years to improve carrier identification, including those based on family history such as the Amsterdam Criteria, clinical prediction models, as well as screening on colon, endometrial, and ovarian tumors. The sensitivity and specificity of these methods, as well as their cost, vary greatly, and importantly, providers must have a high pretest suspicion of Lynch syndrome to employ them effectively. Once an individual is determined to be high-risk, diagnostic testing in the form of germline genetic tests for MMR and *EPCAM* PVs is more straightforward.

2.1 Screening methods to identify individuals at risk for lynch syndrome

As previously stated, there are many tools in existence to identify who should have genetic testing to establish a diagnosis of Lynch Syndrome. Unfortunately, many providers are unaware of these tools and if they do screen patients for hereditary cancer syndromes, they do so based on the classic findings of early age of diagnosis of cancers or multiple Lynch associated cancers in family members over use of validated screening tools (16, 17).

Use of family-history based criteria are the earliest standardized methods proposed for who should be tested for Lynch syndrome. Use of the Amsterdam II Criteria is one of these methods, and recommends testing an individual for Lynch syndrome when they meet all of the following criteria: 1) having 3 relatives with any Lynch-associated cancer with 1 being the first degree relative of the other 2, 2) there are 2 successive generations are affected, and 3) 1 is diagnosed before the age of 50 (18). The sensitivity and specificity of the Amsterdam II Criteria have since been determined to be 25-72% and 78-98%, respectively (19, 20). The low sensitivity is certainly a weakness of this screening method, though a benefit is that it does not require an individual to already be diagnosed with a cancer prior to screening, and considers Lynch-associated cancers other

TABLE 1 Estimated lifetime risks of gynecologic cancers in Lynch syndrome.

Variant	Population EC Risk 3.1%		Population OC Risk 1.3%	
	EC Risk	EC Average Age	OC Risk	OC Average Age
MLH1	34-54%	49	4-20%	46
MSH2/EPCAM	21-57%	47-48	8-38%	43
MSH6	16-49%	53-55	≤1-13%	46
PMS2	13-26%	49-50	1.3-3%	51-59

EC, endometrial cancer; OC, ovarian cancer (12).

than colorectal cancer, which is important for those approaching screening from a women's health perspective. The Amsterdam II Criteria were followed by the Bethesda Criteria and Revised Bethesda Criteria, a set of guidelines based on personal and family history for when patients with colorectal cancer should have their tumors tested for microsatellite instability (MSI), a pathologic hallmark of Lynch-associated cancers (21). The sensitivity and specificity of the Revised Bethesda Criteria were determined to be 50-94% and 25-75% respectively (19, 20). In addition to a lower specificity, from a gynecologic perspective, this screening method is limited by the fact that it requires a colorectal cancer diagnosis and does not take into account endometrial and ovarian malignancies, though the American College of Obstetricians and Gynecologists (ACOG) has made modifications to extrapolate the criteria to gynecologic cancers (22). Furthermore, it is not able to identify individuals with Lynch syndrome prior to a cancer diagnosis. The Society of Gynecologic Oncologists (SGO) also developed guidelines in 2007 that placed individuals within two risk categories (20-25% risk and 5-10% risk) of having Lynch syndrome that were based on the Amsterdam criteria and Bethesda criteria (23). However, follow-up studies show a relatively low sensitivity of SGO criteria consistent with studies of Amsterdam and Bethesda criteria (24, 25).

Clinical prediction models were developed to improve the detection of individuals with Lynch syndrome compared to methods based primarily on family history such as the Amsterdam II Criteria and Revised Bethesda Criteria. Their strength is that they screen for Lynch syndrome prior to a person being diagnosed with cancer. Several models have been created, such as MMRpredict, MMRpro, and PREMM5 (26–28). Each model is somewhat different based on variables they take into account, including characteristics such as age, sex, age at diagnosis of cancer, family history of cancer with family ages of diagnosis, and testing results, if available. However, each is similar in that they are designed to quantify the likelihood a person has a PV in an MMR gene, guiding counseling for the decision to pursue genetic testing. The advantage of these models is that they are simple, validated tools for providers to employ in cancer unaffected individuals that may improve upon the screening test characteristics of the Amsterdam II Criteria and the Revised Bethesda Criteria, though comparative studies are few and conflicting (26, 29, 30). However, it is important to note that not all models quantify risk for PVs in all Lynch-associated genes. Importantly, it should be noted that MMRpredict is validated for patients with colorectal cancer rather than endometrial or ovarian cancers, while MMRpro considers endometrial cancer and PREMM5 considers both endometrial cancer and other Lynch-associated cancers including ovarian cancer (26–28). Gynecologic providers must be aware of this when selecting a clinical prediction model if this is the screening method they choose to utilize.

The current standard of care in screening for Lynch syndrome in those who are affected by cancer is tumor-based testing of patients for loss of expression of MMR proteins with immunohistochemical staining (IHC) (31). IHC staining detects the presence of MMR proteins, and staining is lost when there is a loss or defect in an MMR gene as is seen in Lynch syndrome. This is generally an indication for germline MMR gene testing for diagnosis of Lynch syndrome, though there are additional steps such as *MLH1* hypermethylation testing depending on the pattern of loss of expression visualized to determine whether the loss of expression is sporadic or a result of a germline PV. An adjunct or lesser alternative to IHC staining is tumor-based MSI testing (11). MSI testing is traditionally performed using polymerase chain reaction (PCR) to identify expansion or contraction of repetitive DNA sequences within the tumor that are prone to error, and is recommended when IHC results are equivocal. Tumors that show a certain degree of this expansion or contraction are determined to be MSI-high (MSI-H). Most Lynch tumors are MSI-H, but only about 16% of MSI-H tumors are associated with Lynch syndrome (32). MSI-H tumors are, however, an indication for Lynch genetic testing if identified, regardless of type of malignancy. Tumor testing as a screening method offers the greatest sensitivity and specificity of the methods described, but unfortunately requires a cancer diagnosis for screening to be completed, thus limits primary prevention of cancer in those with Lynch syndrome, though it does offer options for prevention of metachronous malignancies.

When it comes to tumor testing as a method of screening for Lynch syndrome, a key question is knowing which tumors to test. The Revised Bethesda Criteria offer one solution to this question, though may miss 12-30% of Lynch-associated tumors and would need modification and ideally validation for patients with endometrial or ovarian cancer (8). Universal screening of tumors allows the greatest detection of Lynch syndrome, but whether or not it is truly cost-effective remains in question. Studies in colorectal cancer populations support universal colorectal tumor testing as reasonably cost-effective (33). A study in the United Kingdom also found universal IHC staining of endometrial tumors to be cost-effective (34). However, a cost-effectiveness study on a variety of testing criteria in women with endometrial cancer in the United States calculated an incremental cost-effectiveness ratio (ICER) of $648,494 per life year gained for universal endometrial cancer tumor testing, which was significantly greater than $9,126 per year of life gained for the recommended strategy of testing the tumors of all women endometrial cancer with at least 1 first degree relative with Lynch-associated cancer diagnosed at any age (35). Regardless of the cost, because of enhanced detection of Lynch syndrome and prognostic implications of certain molecular subtypes of endometrial carcinomas, universal tumor testing is now recommended by several professional societies, including the National Comprehensive Cancer Network (NCCN), Society of Gynecologic Oncology (SGO), ACOG, and European Society for Medical Oncology (ESMO) (22, 36–38) Whether or not universal endometrial tumor testing as a screening method for Lynch syndrome is performed and the extent of testing may be institution-dependent at this time due to cost and pathology expertise. This poses an issue for equitable care and hopefully further study and technology advances can standardize screening. As for ovarian cancer, NCCN guidelines recommend germline and tumor testing for all patients diagnosed to not only evaluate for Lynch syndrome, but also to evaluate for *BRCA* mutations and

other molecular features that may influence treatment decisions (39). Current guidance from SGO on identifying patients with an increased likelihood of Lynch syndrome takes into account family history as well as molecular based tumor screening techniques (Table 2) (37).

Despite gradual improvements in the detection of individuals at risk for Lynch syndrome, there continues to be a significant number who remain undiagnosed, and development of novel screening strategies or technology to improve access to screening should be a priority. One suggested solution includes use of remote genetic counseling to better identify high-risk individuals who may not have access to in-person genetic counseling, which has been shown to produce similar levels of patient knowledge and satisfaction and reduces costs, but may result in lower counseling and testing completion rates compared to in-person counseling (40, 41). Additionally, genetic counseling itself requires a provider referral, which adds a step in the screening process and therefore adds a barrier. Genetic counseling by providers other than genetic counselors has been explored to remove this barrier, though this is dependent on provider acceptability of and comfortability with performing their own genetic counseling. One recent study evaluated the feasibility of gynecologist led Lynch syndrome counseling and testing, rather than sending a patient to a genetic counselor prior to testing, and results were favorable in terms of acceptability by women being tested and uptake of testing upon counseling (42). Another proposed solution to the genetic counselor barrier suggests use of health information technology in the form of chatbots with which individuals can directly interact (43). These chatbots use an individual's input and standardized risk assessment tools to produce a risk estimate for hereditary cancer syndromes. They can then facilitate genetic testing, counsel regarding results, and assist with cascade testing virtually. One study evaluating use of a chatbot revealed patient knowledge and genetic testing completion rates similar to those achieved through genetic counseling, but low rates of individuals initiating interaction with the chatbot and low provider interest or comfort in results follow-up (44). Studies evaluating patient and provider acceptability of chatbots and process implementation are ongoing. Population-based genetic testing for germline PVs has also been proposed given improvements in DNA sequencing and lower costs (45). Indeed, this method identifies many individuals with hereditary cancer syndromes who would not otherwise meet high-risk criteria, but is not cost-effective at this time, and the stress of finding variants of unknown significance (VUS) may adversely affect some individuals.

2.2 Diagnosis of lynch syndrome

Once a person has been screened as high-risk for Lynch syndrome using one of the methods above, the definitive diagnosis can be established through germline testing. This can be done through several methods: multigene panel testing, targeted MMR gene testing, or single gene testing. The National Comprehensive Cancer Network (NCCN) recommends single gene testing if there is a known PV within a person's family and if they are clinically low-risk for other PVs (46). Multigene panel testing, in which several cancer predisposition PVs are sequenced, is recommended for those at high-risk for Lynch syndrome but without a known PV in the family due to the possibility of another hereditary cancer syndrome placing the individual in the high-risk category. MMR PV testing may be best for those with a specific IHC pattern after tumor testing. Multiple professional societies, including ACOG and NCCN, agree that it is best practice to involve cancer genetics experts, such as genetic counselors, whenever genetic testing may be performed. However, given a national shortage of these skilled professionals, this may not be a resource for all, and presents an area for improved access for equitable high-risk care (22, 46).

Interpretation of genetic testing results is key to counseling (Table 3) (47). VUS are changes or alterations in the genetic code for which the downstream protein function is unknown and are sometimes the most clinically challenging result to contextualize for patients. As there is increased utilization of multigene panels in broader populations, more VUSs will be identified (48). Approximately 80-90% of VUSs will subsequently be reclassified as benign polymorphisms and should be treated as clinically negative (49). In addition, an increasing number of individuals present to care having completed direct to consumer testing (DTC), where these individuals interact directly with testing companies. There is a wide range of DTC companies with different testing methodology and interpretation of results. Currently, any PV identified on DTC should be verified through a clinical lab (47). Lastly, there are families that meet Amsterdam criteria and a germline PV is not identified in the family. These individuals may be followed as having clinical Lynch syndrome, however, this should be done in consultation with a high-risk expert.

Once an individual is diagnosed with Lynch syndrome, they should be counseled regarding both their personal risk of cancer and also their family's potential risk. This should include a conversation regarding cascade testing, which involves genetic testing of a known carrier's relatives to determine whether these family members are affected, and thus also at increased risk.

TABLE 2 Patients at increased risk of LS for whom genetic assessment is recommended (modified from SGO statement on risk assessment for inherited gynecologic cancer predispositions).

Patients with EC or CRC with evidence of MSI or loss of DNA MMR protein (MLH1, MSH2, MSH6, PMS2) on IHC.
Patients with FDR affected with EC or CRC diagnosed <60 years or identified to be at risk for LS by systematic clinical screen that incorporated focused personal and medical history.
Patients with FDR or SDR with a known pathogenic variant in a MMR gene.

EC, endometrial cancer; CRC, colorectal cancer; MSI, microsatellite instability; MMR, mismatch repair; FDR, first degree relative; LS, Lynch syndrome; SDR, second degree relative; IHC, immunohistochemistry (37).

TABLE 3 Interpretation of germline genetic testing results (47).

Result	Description
True Positive	Individual is a carrier of an alteration in a known cancer-predisposing gene
True Negative	Individual is not a carrier of a known cancer-predisposing gene that has been positively identified in another family member
Indeterminate	Individual is not a carrier of a known cancer-predisposing gene and carrier status of other family members is also negative or unknown
Variant of Unknown Significance	Individual is a carrier of gene alteration that currently has no known significance

Cascade testing is recommended to begin with first-degree relatives, as these individuals have a 50% chance of having the same Lynch syndrome PV, and if positive, expand cascade testing to their first-degree relatives (50). Generally, these individuals need only be tested for the known PV that has been identified in their family rather than testing for all PVs associated with Lynch syndrome. Cascade testing enhances the ability to identify more carriers with Lynch syndrome that otherwise might not be screened, and improves the cost-effectiveness of universal tumor testing (51).

Another consideration to offer individuals interested in reproduction upon diagnosis of Lynch syndrome is referral to a reproductive endocrinology and infertility (REI) specialist to discuss preimplantation genetic testing (PGT). This diagnostic test is used in conjunction with *in vitro* fertilization (IVF), and involves testing an embryo in the lab for a specific PV to reduce the risk of passing this PV on to future children. For Lynch syndrome, this would mean testing the embryos of a Lynch syndrome carrier and their partner for the specific MMR PV the carrier is known to have. Identifying which embryos carry this PV allows the REI specialist to inform the parents undergoing IVF and selectively transfer embryos that are not carriers, thus preventing a future child from being affected by Lynch syndrome. It is important to address the timing of IVF if the Lynch syndrome carrier is female, as it may be affected by the recommended timing of risk-reducing hysterectomy to prevent endometrial cancer or risk-reducing oophorectomy to prevent ovarian cancer, as is discussed in the next section (52).

3 Gynecologic cancer screening and risk reduction in individuals with lynch syndrome

Once a diagnosis of Lynch syndrome is established, it is recommended to begin the process of screening for early development of Lynch-associated cancers and in some instances undergo risk-reducing procedures or initiate chemoprevention under the care of physicians with expertise in the management of high-risk carriers. While screening and risk reduction methods exist for other Lynch-associated cancers, colonoscopy screening for colorectal cancer being at the forefront, this review will focus on those measures targeted towards the screening and prevention of endometrial and ovarian cancers in those with Lynch syndrome.

3.1 Endometrial cancer

Multiple approaches to screening for endometrial cancer in asymptomatic women diagnosed with Lynch syndrome have been proposed, including endometrial biopsy and transvaginal ultrasound (TVUS). Importantly, none of these methods have been shown to reduce the morbidity and mortality of women with Lynch syndrome (53–56). This is likely due to the fact that the majority of endometrial cancers are already diagnosed with early stage disease and any screening intervention will not improve dramatically on the early stage of diagnosis overall for endometrial cancer. Despite a lack of proven efficacy, and given the low risk of screening tests and high risk of endometrial cancer in this population, multiple professional societies including ACOG and NCCN agree that endometrial biopsy every 1-2 years starting between the ages of 30 and 35 can be considered in women diagnosed with Lynch syndrome (22, 46). Many experts go on to recommend starting screening with endometrial biopsy 10 years before the earliest Lynch-associated cancer diagnosis in the family and to continue endometrial biopsies until the time of hysterectomy. Endometrial biopsy is the test of choice due to its excellent sensitivity of 91-99.6% and specificity of 98% for endometrial cancer and hyperplasia, as well as evidence that it enhances detection of endometrial cancer and hyperplasia compared to TVUS alone (54, 57). It is, however, an invasive and uncomfortable test, which may impact acceptability to patients. There is prospective evidence from patient reported outcomes that performing endometrial biopsy at the time of colonoscopy decreased pain associated with the biopsy (58). While this was shown to be feasible in the setting of a study, whether this is feasible in practice depends on many factors, most notably where colonoscopies are performed within individual practices.

TVUS is less invasive than endometrial biopsy, however, it offers lower detection rates and it is not recommended in premenopausal females since endometrial thickness varies greatly throughout a menstrual cycle (46, 54). A few studies have investigated the combination of endometrial biopsy and TVUS, which shows promise in increasing detection, but again, does not offer definitive morbidity or mortality benefit at this time (55, 59).

Additional methods of screening for endometrial cancer have been proposed and show potential, but currently lack sufficient evidence supporting efficacy required of a suitable screening test in a clinical setting. The use of pap smears and tampon-based intravaginal sampling to detect cancerous endometrial cells, shed

tumor DNA, or biomarkers are a few of these methods (60–63). One such study evaluating 18 genes in fluid collected *via* pap smear for endometrial and ovarian cancer patients in comparison to cancer-free controls found a specificity of 99%, though it was limited by modest sensitivity (78% for endometrial cancer, 33% for ovarian cancer) (63). Analysis of urinary samples for potential endometrial cancer biomarkers, such as estrogen metabolites, has also been proposed by several studies and is interesting as a simple, non-invasive form of screening, but to date none of the biomarkers suggested have been validated (64–66). Each of these will be ideas to watch over the next several years.

For women diagnosed with Lynch syndrome who present with symptoms, including but not limited to abnormal bleeding, pelvic mass or pain, abnormal discharge, or weight loss, women's health providers should have high suspicion for endometrial cancer and investigation in the form of endometrial biopsy and/or transvaginal ultrasound should be performed.

Risk reduction of endometrial cancer is another aspect of management of which providers and women with Lynch syndrome need to be aware (Table 4). The most invasive method, and that with the greatest evidence for prevention of endometrial cancer in this population, is risk-reducing hysterectomy and bilateral salpingectomy with or without oophorectomy. The largest study comparing outcomes between women with Lynch syndrome who underwent risk-reducing surgery and those who did not found that 0% of patients who underwent hysterectomy were diagnosed with endometrial cancer after 13 years of follow-up compared to 33% of those who did not have a hysterectomy (14). The timing of this intervention is controversial given lack of strong evidence dictating a specific age, though desire for fertility, age at cancer diagnosis in Lynch-affected family members, diagnosis of other cancers, and even specific PV should all be considered (46). Given a 4-fold increase in endometrial cancer risk from the age of 40 to the age of 50, many professional societies, including ACOG and SGO, recommend risk-reducing hysterectomy by the age of 40-45 (4, 22). Surgery before age 40 can also be considered if a woman has completed child-bearing, and indeed, the American Society for Clinical Oncology (ASCO) recommends this (67). A cost-effectiveness analysis comparing multiple ages for surgery found that annual screening until hysterectomy at age 40 was most effective at preventing endometrial cancer, but risk-reducing surgery at age 40 without screening was the most cost-effective given the substantial cost of screening and hormone replacement therapy for surgical menopause when surgery was performed at age 30 (68). Despite these results, expert consensus is to continue screening for endometrial cancer until hysterectomy is performed, at which point it can be discontinued. For those desiring fertility, referral to a reproductive endocrinologist should be considered prior to surgery, especially if they are approaching advanced maternal age. If colorectal cancer is diagnosed prior to risk-reducing gynecologic surgery, a joint procedure with colorectal surgery can be planned and this can also dictate timing of surgery. Regardless of timing, colonoscopy and endometrial biopsy should be up to date prior to risk-reducing surgery to rule out occult malignancies and be sure the appropriate procedure is being planned.

Lifestyle and medical chemoprevention options should also be discussed with individuals with Lynch syndrome. In accordance with general population recommendations, those with Lynch syndrome should be counselled to maintain or attain a normal body weight given the well-defined association of obesity with the development of endometrial cancer. They should also be counselled to engage in 30 minutes of exercise daily or 150-300 minutes of moderate intensity exercise weekly exercise per American Cancer Society (ACS) guidelines (69).

As for medical chemoprevention, there is evidence that daily aspirin may be associated with a reduction in all Lynch-associated cancer diagnoses in those with Lynch syndrome (70). The CAPP2

TABLE 4 Expert screening and risk reduction recommendations for gynecologic cancers in Lynch syndrome, modified from NCCN Guidelines (Version 2.2022: Lynch Syndrome) (12).

Variant	EC Screening and Risk Reduction	OC Screening and Risk Reduction
MLH1	EBx every 1-2 years starting at 30-35 can be considered Consider RR agents Hysterectomy as RR option can be considered, timing based on patient factors	TVUS and CA125 may be considered at clinician's discretion Consider RR agents BSO as a RR option should be individualized
MSH2/ EPCAM	EBx every 1-2 years starting at 30-35 can be considered Consider RR agents Hysterectomy as RR option can be considered, timing based on patient factors	TVUS and CA125 may be considered at clinician's discretion Consider RR agents BSO as a RR option should be individualized
MSH6	EBx every 1-2 years starting at 30-35 can be considered Consider RR agents Hysterectomy as RR option can be considered, timing based on patient factors	TVUS and CA125 may be considered at clinician's discretion Consider RR agents Insufficient evidence for specific recommendation, BSO as a RR option should be individualized
PMS2	EBx every 1-2 years starting at 30-35 can be considered Consider RR agents *PMS2* carriers appear to be at only a modestly increased risk of EC: Hysterectomy as RR option can be considered, timing based on patient factors	TVUS and CA125 may be considered at clinician's discretion Consider RR agents Insufficient evidence for specific recommendation, *PMS2* carriers appear to be at no greater than average risk, BSO as a RR option should be individualized in consultation with gynecologist with expertise in LS

Note that these are the recommendations of NCCN, and that recommendations may vary depending on the professional organization. EC, endometrial cancer; OC, ovarian cancer; EBx, endometrial biopsy; RR, risk reducing; BSO, bilateral salpingo-oophorectomy; TVUS, transvaginal ultrasound.

trial is a multinational randomized controlled trial examining differences in colorectal and all Lynch cancer diagnoses in those with Lynch syndrome based on use of daily aspirin. Individuals were randomized to receive either placebo or 600mg of aspirin daily. After an average of 10 years of follow-up, colorectal cancer and all Lynch cancer diagnoses were found to be significantly lower in those taking daily aspirin, though there was no difference noted for all non-colorectal Lynch cancer diagnoses. While the benefit for endometrial or ovarian cancers is unclear, there is benefit for colorectal cancer and thus women with Lynch syndrome can be offered daily aspirin for their comprehensive care if there are no contraindications.

Hormonal therapies, including combined oral contraceptive pills (OCPS), oral progestins, or progesterone containing intrauterine devices (IUDs), are an alternative form of risk-reduction for those with Lynch syndrome prior to completion of childbearing and risk-reducing hysterectomy. Data for endometrial cancer prevention with hormonal therapies in the Lynch population specifically are limited and the majority of evidence is extrapolated from studies in the general population (71–73). Population based evidence shows that OCP use for 5 years decreases endometrial cancer risk by 50-70%, with increased protection with longer duration of treatment (74). Similarly, retrospective evaluation showed a 61% risk reduction in endometrial cancer among women with Lynch syndrome who used hormonal contraception in the form of combined or progestin only pill, the implant, or the injection for at least one year (75). A small randomized controlled trial examined the effect of Depo-Provera versus progestin-only oral contraceptives in a population of women with Lynch syndrome, which revealed a decrease in endometrial proliferation in both groups, but was not able to compare endometrial cancer rates between the groups (76). Progesterone containing IUDs, which are now utilized to treat endometrial intraepithelial neoplasia and low grade endometrial cancers, are also associated with an approximate 50% decreased risk of endometrial cancer in the general population and this decreased risk persists for 5 year following discontinuation (77–79).

3.2 Ovarian cancer

As with endometrial cancer, multiple screening methods for the early detection of ovarian cancer have been proposed for asymptomatic women with Lynch syndrome, though no evidence exists supporting an improvement in morbidity or mortality for any method (55, 56). Annual transvaginal ultrasound is one method that has been studied, but has relatively poor sensitivity and specificity for ovarian cancer and thus can be considered, but is not formally recommended by major professional gynecologic or oncology professional organizations such as ACOG, SGO, or NCCN (22, 46, 80). The same is true for the measurement of CA 125. Importantly, there are no studies on these screening methods in the Lynch syndrome population specifically, they are only available from the general population or among those with BRCA mutations (81–84). This is problematic because Lynch-associated ovarian cancer is different from BRCA-associated ovarian cancer. Lynch associated ovarian cancers tend to be mostly endometrioid and have a more favorable prognosis than the aggressive serous ovarian cancers associated with BRCA mutations (85). They also derive from different molecular pathways. Thus, combining these two very different types of ovarian cancers under one umbrella based on evidence availability, or lack thereof, should be done with great caution. Certainly, more studies in a Lynch population are needed.

Any female with Lynch syndrome presenting with bloating, a palpable mass, abdominal pain, weight gain or loss, early satiety, or other concerning symptoms should undergo imaging to determine if ovarian cancer is present.

Risk-reducing surgery for the prevention of ovarian cancer in those with Lynch syndrome is currently one of the most difficult clinical questions to consider in high-risk care for the individual as differential lifetime risks of ovarian cancer for specific Lynch variants have been better outlined in recent years (Table 4). Most data evaluating risk reduction for ovarian cancer include bilateral salpingo-oophorectomy (BSO) with tubes and ovaries removed at the same time and includes data for all variants in aggregate, rather than for individual variants. This is critical to understand for counselling, especially those with PV in *MSH6* and *PMS2*, where there is no strong recommendation for oophorectomy based on current available evidence. It should be noted throughout the discussion that regardless of the recommendation for bilateral oophorectomy, bilateral salpingectomy is recommended at the time of risk reducing hysterectomy (86, 87).

NCCN currently recommends for *MLH1* and *MSH2/EPCAM* carriers that the decision to have a BSO as a risk-reducing option should be individualized and timing should be based on completion of childbearing, menopausal status, medical comorbidities, family history, and specific variant. Differently, NCCN states that for *MSH6* carriers, insufficient evidence exists to make a specific recommendation for BSO and that the decision should be individualized. They are even more detailed in their recommendation for *PMS2* carriers and state that *PMS2* carriers appear to be at no greater risk of ovarian cancer and that individuals may reasonably elect not to have an oophorectomy (12).

In a study evaluating BSO compared to no BSO for the prevention of ovarian cancer in 223 individuals with Lynch syndrome, no one who underwent BSO was diagnosed with ovarian cancer, while 5% who did not undergo BSO were ultimately diagnosed with ovarian cancer, supporting BSO as a reasonable method of risk reduction (14). Ovarian cancer risk in those with Lynch syndrome triples from the age of 40 to the age of 50 depending on the PV, therefore timing recommendations of surgery before age 45 are the similar to endometrial cancer (4). Hormone replacement therapy (HRT) is generally considered safe for the treatment of surgical menopause after BSO in premenopausal women, though has not been directly studied in a Lynch population. Given that hysterectomy is also recommended for risk reduction, women benefit from needing estrogen replacement therapy (ERT) alone. Expert opinion is for consideration of HRT for women with Lynch Syndrome who undergo premenopausal BSO (46). Interestingly, there is evidence of a protective effect of HRT against the development of colorectal cancer in the general population, and this may be helpful for women with

Lynch syndrome who have an increased risk of colorectal cancer, though further study in this specific population would be needed (88).

For the individual, discussion of the risks and benefits of oophorectomy is paramount in the Lynch population and should include a gynecologic provider experienced in high-risk care. For most, the decision is whether to proceed with oophorectomy at the time of hysterectomy and bilateral salpingectomy. Several factors should be taken into account on top of completion of childbearing, most notably age, family history of ovarian cancer and age of diagnosis, as well as other medical and surgical co-morbidities. Regardless of the individual variant, if an individual is ready to proceed with risk reducing hysterectomy at 35, a well-documented and thorough discussion of oophorectomy is necessary, most notably including the risk of early surgical menopause, including increased risk of osteoporosis, cardiovascular disease, and effects on cognitive as well as sexual function. In addition, there is mounting evidence that the majority of high grade serous ovarian cancers originate in the distal fallopian tube. Opportunistic salpingectomy is associated with a 42-64% reduction in the risk of ovarian cancer in epidemiologic studies (89–91). There are ongoing studies in the BRCA population for this, but the degree to which salpingectomy decreases the risk of Lynch associated ovarian cancers specifically is largely unknown, where the incidence of non-serous ovarian cancers are higher than in the BRCA population (92). Delayed oophorectomy is an option for those who wish to defer menopause with appropriate counselling, though this would require a second surgery if hysterectomy is done earlier and surgery thus may be more complicated (93).

Ovarian cancer chemoprevention with combined estrogen and progestin oral contraceptive pills (COCPs) is an option for women with Lynch syndrome, though again, there are no studies in the Lynch syndrome population. Studies in the general or BRCA populations do suggest a benefit in ovarian cancer prevention with the use of COCPs, but again, Lynch-associated ovarian cancer is fundamentally different than BRCA-associated ovarian cancer, thus it is not clear whether a true benefit exists in the Lynch syndrome population (46, 94–97). Premenopausal females with Lynch syndrome who have not completed childbearing and thus have not yet undergone risk-reducing hysterectomy and BSO can have a risk/benefit discussion with their provider to determine if chemoprevention with COCPs is the right choice for them.

An exciting intervention that may be on the horizon for cancer prevention in carriers of Lynch syndrome is that of cancer vaccines. These investigational vaccines are developed against neoantigens produced by frameshift mutations in those with MSI-H tumors and Lynch syndrome (98). There are currently multiple registered clinical trials investigating the development of vaccines to prevent cancer in those with Lynch syndrome specifically (99).

4 Treatment of gynecologic cancer in patients with lynch syndrome

All treatment options that are available to those with sporadic endometrial and ovarian cancers are also available to those with Lynch-associated endometrial and ovarian cancers, and prior to molecular analysis of tumors, treatment recommendations were the same regardless of Lynch status. Since the relatively recent discovery that MMR deficient and MSI-H tumors, the key characteristics of Lynch tumors, may be more susceptible to immunotherapy that functions *via* PD-1 blockade than tumors without these features, there is now evidence that patients with Lynch-associated tumors may benefit from alternative treatment plans (100). It is worthwile to note here that MSI-H tumors and MMR deficient tumors that arise sporadically may have a different prognosis than those that arise due to Lynch syndrome, and therefore applying research on treatments in all MMR-deficient tumors to Lynch syndrome must be done with caution, though studies in the Lynch syndrome population alone are limited (9).

Whether initial adjuvant therapy for endometrial cancer should be dictated by MMR deficiency is controversial based on available evidence. A retrospective study comparing outcomes between MMR deficient endometrial tumors and MMR proficient endometrial tumors following adjuvant therapy with either radiation or chemotherapy revealed a trend toward lower recurrence rates among patients with MMR deficiency, but on multivariate analysis, there was no association with progression-free or overall survival (101). However, a separate retrospective study found improved survival when patients with MMR-deficient endometrial cancer were treated with radiation therapy compared to those who were MMR-proficient (102). A third retrospective study found worse recurrence-free survival after vaginal brachytherapy in those with MMR-deficient endometrial cancer compared to those with MMR proficiency (103). When comparing chemoradiation to radiation therapy alone as adjuvant therapy for MMR deficient tumors of women in the PORTEC-3 population, no benefit was found with the addition of chemotherapy (104). Other studies are underway to investigate the influence of various adjuvant treatments on MMR deficient tumors, such at the MMRd-GREEN Trial under the RAINBO program, which is prospectively examining recurrence-free survival between patients with MMR deficient high-risk endometrial tumors randomized to receiving either radiation therapy alone or durvalumab, an immunotherapeutic, with radiation therapy (105). Furthermore, both PORTEC-4a and a clinical trial from China are currently investigating adjuvant therapies for early stage endometrial cancer based on either molecular classification (such as MMR-deficient) or traditional risk stratification (106, 107).

Regarding radiation therapy in those with Lynch-associated endometrial cancer, one should also consider the possibility of second primary malignancies after radiation treatment. While evidence is unavailable for an increased risk of second primaries attributable to radiation treatment for endometrial cancer specifically in a Lynch population, there is some evidence of increased risk for second primary malignancies after radiation for endometrial cancer in the general population (108). Other studies have found no increased risk of second primary malignancies attributable to radiation therapy for endometrial cancer (109). Insufficient evidence exists for formal recommendations, but if an association exists between radiation therapy for endometrial cancer and second primary malignancies, those at increased risk of second

primary malignancies in the first place such as those with Lynch syndrome may need to be approached more cautiously with radiation therapy, and at the very least continue close surveillance with regular colonoscopies. Close communication between a patient's gastroenterology and oncology teams is warranted in this situation.

Evidence regarding initial adjuvant therapy in MMR-deficient ovarian cancers is also conflicting. One retrospective study found similar survival rates between MMR-deficient and MMR-proficient ovarian cancers, thus recommended treating them similarly (13). Some studies report improved survival in MMR-deficient cases compared to MMR-proficient cases that could be considered when deciding on therapy, but not all took into account a higher likelihood of endometrioid histology associated with MMR deficiency, which is a significant prognostic factor (110–112). Another study reported improved survival in patients with high expression of MMR genes who were treated with platinum-based chemotherapy, supporting *in vitro* studies that called into question whether platinum resistance is associated with MMR deficiency (110, 113). At this time, given conflicting and limited evidence, there are no societal recommendations regarding MMR deficiency and initial adjuvant treatment in either endometrial or ovarian cancer. Further study is needed.

While studies are inconclusive regarding MMR deficiency and its influence on upfront therapy, recent evidence in favor of immunotherapy for recurrent or progressive MMR-deficient endometrial and ovarian cancers has emerged. The theory behind immunotherapy is to utilize the body's own immune system to attack tumor cells, and this branch of treatment has been shown to be effective in multiple types of cancer. In fact, pembrolizumab, a monoclonal antibody that inhibits T-cell apoptosis by blocking the PD-1 receptor on these immune cells, is FDA approved for all non-colorectal, MSI-H and MMR deficient tumors, regardless of tumor site, and it the first therapy to receive accelerated approval for a tumor-agnostic indication (114). In gynecologic cancer specifically, pembrolizumab is the immunotherapeutic best studied and supported (100, 115). The largest trial on this topic presently is the Keynote 158 study, which published results of a phase II randomized controlled trial evaluating the efficacy of pembrolizumab in the treatment of non-colorectal, MSI-H and MMR-deficient tumors (116). The study included 49 cases of endometrial cancer and 15 cases of ovarian cancer. Results revealed a 34.3% objective response rate, supporting pembrolizumab as a treatment option in this population. NCCN now recommends pembrolizumab for the treatment of MSI-H and MMR deficient endometrial and ovarian tumors that fail to respond adequately to first-line therapy and this recommendation should be discussed with patients with Lynch syndrome (38, 39).. More clinical trials are underway evaluating immunotherapy among women with MMR-deficient advanced or recurrent endometrial tumors, such as the KEYNOTE-C93 trial investigating pembrolizumab versus platinum-doublet chemotherapy and the DOMENICA trial investigating dostarlimab, another immunotherapeutic, versus platinum-doublet chemotherapy (117, 118).

Whether immunotherapy will be of use for upfront treatment of Lynch-associated endometrial or ovarian cancers is under investigation. Not only will the MMRd-GREEN Trial shed light on this, there is also the IMHOTEP trial, which is currently underway investigating pembrolizumab as neoadjuvant therapy prior to surgical resection of MSI-H and MMR-deficient tumors of multiple sites (119). The use of immunotherapy in the neoadjuvant setting for MSI-H and MMR-deficient colorectal cancer has been reported with promising results in several case studies (120–122), and multiple clinical trials studying neoadjuvant immunotherapy are underway in this population. It will be exciting to monitor progress in this field over the next few years to determine whether neoadjuvant immunotherapy can have a similar impact on MMR-deficient endometrial and ovarian cancers.

5 Conclusion

While much has been discovered about our understanding of cancer risk and our ability to reduce risk for those with Lynch syndrome, there remains a great deal to be discovered to diminish its associated morbidity and mortality. Improvements in technology are needed to increase identification of individuals at high risk for Lynch syndrome, not only by utilizing high quality screening tests, but also for increased patient access to these tools and for reduction in costs to allow more universal testing. The same is true for screening for Lynch-associated cancers in patients diagnosed with Lynch syndrome, especially given that endometrial cancer and ovarian cancer screening has not yet been shown to have a mortality benefit. Identifying barriers and improving access to risk reduction measures is another future direction in the study of Lynch syndrome, and perhaps the greatest frontier is determining whether Lynch-associated endometrial and ovarian tumors should be treated differently than sporadic endometrial and ovarian tumors. Dedication to these efforts will bring about the implementation of important practice changes and hopefully afford us the mortality benefit in the management of Lynch-associated endometrial and ovarian cancers we have been seeking.

Author contributions

KU was involved in concept development, writing, and editing. KR was involved in concept development, writing, and editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Boland CR, Lynch HT. The history of lynch syndrome. *Fam Cancer.* (2013) 12 (2):145–57. doi: 10.1007/s10689-013-9637-8

2. Win AK, Jenkins MA, Dowty JG, Antoniou AC, Lee A, Giles GG, et al. Prevalence and penetrance of major genes and polygenes for colorectal cancer. *Cancer Epidemiol Biomarkers Prev* (2017) 26(3):404–12. doi: 10.1158/1055-9965.EPI-16-0693

3. Dowty JG, Win AK, Buchanan DD, Lindor NM, Macrae FA, Clendenning M, et al. Cancer risks for MLH1 and MSH2 mutation carriers. *Hum Mutat* (2013) 34 (3):490–7. doi: 10.1002/humu.22262

4. Bonadona V, Bonaïti B, Olschwang S, Grandjouan S, Huiart L, Longy M, et al. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in lynch syndrome. *JAMA* (2011) 305(22):2304–10. doi: 10.1001/jama.2011.743

5. Dominguez-Valentin M, Sampson JR, Seppälä TT, Ten Broeke SW, Plazzer JP, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: Findings from the prospective lynch syndrome database. *Genet Med* (2020) 22(1):15–25. doi: 10.1038/s41436-019-0596-9

6. Watson P, Vasen HFA, Mecklin JP, Bernstein I, Aarnio M, Järvinen HJ, et al. The risk of extra-colonic, extra-endometrial cancer in the lynch syndrome. *Int J Cancer.* (2008) 123(2):444–9. doi: 10.1002/ijc.23508

7. Lu KH, Dinh M, Kohlmann W, Watson P, Green J, Syngal S, et al. Gynecologic cancer as a "sentinel cancer" for women with hereditary nonpolyposis colorectal cancer syndrome. *Obstet Gynecol.* (2005) 105(3):569–74. doi: 10.1097/01.AOG.0000154885.44002.ae

8. Moreira L, Balaguer F, Lindor N, de la Chapelle A, Hampel H, Aaltonen LA, et al. Identification of lynch syndrome among patients with colorectal cancer. *Jama* (2012) 308(15):1555–65. doi: 10.1001/jama.2012.13088

9. Post CCB, Stelloo E, Smit V, Ruano D, Tops CM, Vermij L, et al. Prevalence and prognosis of lynch syndrome and sporadic mismatch repair deficiency in endometrial cancer. *J Natl Cancer Inst* (2021) 113(9):1212–20. doi: 10.1093/jnci/djab029

10. Ryan NAJ, Glaire MA, Blake D, Cabrera-Dandy M, Evans DG, Crosbie EJ. The proportion of endometrial cancers associated with lynch syndrome: a systematic review of the literature and meta-analysis. *Genet Med* (2019) 21(10):2167–80. doi: 10.1038/s41436-019-0536-8

11. Ryan NAJ, McMahon R, Tobi S, Snowsill T, Esquibel S, Wallace AJ, et al. The proportion of endometrial tumours associated with lynch syndrome (PETALS): A prospective cross-sectional study. *PloS Med* (2020) 17(9):e1003263. doi: 10.1371/journal.pmed.1003263

12. *National Comprehensive Cancer Network. Genetic/Familial High-Risk Assessment: Colorectal* (Version 2.2022) (2022). Available from: https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf.

13. Crijnen TE, Janssen-Heijnen ML, Gelderblom H, Morreau J, Nooij MA, Kenter GG, et al. Survival of patients with ovarian cancer due to a mismatch repair defect. *Fam Cancer.* (2005) 4(4):301–5. doi: 10.1007/s10689-005-6573-2

14. Schmeler KM, Lynch HT, Chen LM, Munsell MF, Soliman PT, Clark MB, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the lynch syndrome. *N Engl J Med* (2006) 354(3):261–9. doi: 10.1056/NEJMoa052627

15. Hampel H, de la Chapelle A. The search for unaffected individuals with lynch syndrome: do the ends justify the means? *Cancer Prev Res (Phila)* (2011) 4(1):1–5. doi: 10.1158/1940-6207.CAPR-10-0345

16. Christianson CA, Powell KP, Hahn SE, Blanton SH, Bogacik J, Henrich VC. The use of a family history risk assessment tool within a community health care system: Views of primary care providers. *J Genet Couns.* (2012) 21(5):652–61. doi: 10.1007/s10897-011-9479-1

17. Vig HS, Armstrong J, Egleston BL, Mazar C, Toscano M, Bradbury AR, et al. Cancer genetic risk assessment and referral patterns in primary care. *Genet Test Mol Biomarkers.* (2009) 13(6):735–41. doi: 10.1089/gtmb.2009.0037

18. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, lynch syndrome) proposed by the international collaborative group on HNPCC. *Gastroenterology* (1999) 116(6):1453–6. doi: 10.1016/S0016-5085(99)70510-X

19. Tranø G, Sjursen W, Wasmuth HH, Hofsli E, Vatten LJ. Performance of clinical guidelines compared with molecular tumour screening methods in identifying possible lynch syndrome among colorectal cancer patients: A Norwegian population-based study. *Br J Cancer.* (2010) 102(3):482–8. doi: 10.1038/sj.bjc.6605509

20. Syngal S, Fox EA, Eng C, Kolodner RD, Garber JE. Sensitivity and specificity of clinical criteria for hereditary non-polyposis colorectal cancer associated mutations in MSH2 and MLH1. *J Med Genet* (2000) 37(9):641–5. doi: 10.1136/jmg.37.9.641

21. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* (2004) 96(4):261–8. doi: 10.1093/jnci/djh034

22. American College of Obstetricians and GynecologistsACOG practice bulletin no. 147: Lynch syndrome. *Obstet Gynecol.* (2014) 124(5):1042–54.

23. Lancaster JM, Powell CB, Kauff ND, Cass I, Chen L-M, Lu KH, et al. Society of gynecologic oncologists education committee statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecologic Oncol* (2007) 107(2):159–62. doi: 10.1016/j.ygyno.2007.09.031

24. Bruegl AS, Djordjevic B, Batte B, Daniels M, Fellman B, Urbauer D, et al. Evaluation of clinical criteria for the identification of lynch syndrome among unselected patients with endometrial cancer. *Cancer Prev Res* (2014) 7(7):686–97. doi: 10.1158/1940-6207.CAPR-13-0359

25. Ring KL, Bruegl AS, Allen BA, Elkin EP, Singh N, Hartman A-R, et al. Germline multi-gene hereditary cancer panel testing in an unselected endometrial cancer cohort. *Modern Pathology.* (2016) 29(11):1381–9. doi: 10.1038/modpathol.2016.135

26. Barnetson RA, Tenesa A, Farrington SM, Nicholl ID, Cetnarskyj R, Porteous ME, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. *N Engl J Med* (2006) 354(26):2751–63. doi: 10.1056/NEJMoa053493

27. Balmaña J, Stockwell DH, Steyerberg EW, Stoffel EM, Deffenbaugh AM, Reid JE, et al. Prediction of MLH1 and MSH2 mutations in lynch syndrome. *JAMA* (2006) 296 (12):1469–78. doi: 10.1001/jama.296.12.1469

28. Chen S, Wang W, Lee S, Nafa K, Lee J, Romans K, et al. Prediction of germline mutations and cancer risk in the lynch syndrome. *JAMA* (2006) 296(12):1479–87. doi: 10.1001/jama.296.12.1479

29. Balmaña J, Balaguer F, Castellví-Bel S, Steyerberg EW, Andreu M, Llor X, et al. Comparison of predictive models, clinical criteria and molecular tumour screening for the identification of patients with lynch syndrome in a population-based cohort of colorectal cancer patients. *J Med Genet* (2008) 45(9):557–63. doi: 10.1136/jmg.2008.059311

30. Tresallet C, Brouquet A, Julié C, Beauchet A, Vallot C, Ménégaux F, et al. Evaluation of predictive models in daily practice for the identification of patients with lynch syndrome. *Int J Cancer.* (2012) 130(6):1367–77. doi: 10.1002/ijc.26144

31. McConechy MK, Talhouk A, Li-Chang HH, Leung S, Huntsman DG, Gilks CB, et al. Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas. *Gynecol Oncol* (2015) 137(2):306–10. doi: 10.1016/j.ygyno.2015.01.541

32. Latham A, Srinivasan P, Kemel Y, Shia J, Bandlamudi C, Mandelker D, et al. Microsatellite instability is associated with the presence of lynch syndrome pan-cancer. *J Clin Oncol* (2019) 37(4):286–95. doi: 10.1200/JCO.18.00283

33. Mvundura M, Grosse SD, Hampel H, Palomaki GE. The cost-effectiveness of genetic testing strategies for lynch syndrome among newly diagnosed patients with colorectal cancer. *Genet Med* (2010) 12(2):93–104. doi: 10.1097/GIM.0b013e3181cd666c

34. Snowsill TM, Ryan NAJ, Crosbie EJ. Cost-effectiveness of the Manchester approach to identifying lynch syndrome in women with endometrial cancer. *J Clin Med* (2020) 9(6):1664. doi: 10.3390/jcm9061664

35. Kwon JS, Scott JL, Gilks CB, Daniels MS, Sun CC, Lu KH. Testing women with endometrial cancer to detect lynch syndrome. *J Clin Oncol* (2011) 29(16):2247–52. doi: 10.1200/JCO.2010.32.9979

36. Colombo N, Creutzberg C, Amant F, Bosse T, González-Martín A, Ledermann J, et al. ESMO-ESGO-ESTRO consensus conference on endometrial cancer: Diagnosis, treatment and follow-up. *Ann Oncol* (2016) 27(1):16–41. doi: 10.1093/annonc/mdv484

37. Lancaster JM, Powell CB, Chen LM, Richardson DL. Society of gynecologic oncology statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecol Oncol* (2015) 136(1):3–7. doi: 10.1016/j.ygyno.2014.09.009

38. National Comprehensive Cancer Network. *Uterine neoplasms (Version 1.2023)* (2022). Available at: https://www.nccn.org/professionals/physician_gls/pdf/uterine.pdf.

39. Armstrong DK, Alvarez RD, Backes FJ, Bakkum-Gamez JN, Barroilhet L, Behbakht K, et al. NCCN guidelines® insights: Ovarian cancer, version 3.2022. *J Natl Compr Canc Netw* (2022) 20(9):972–80. doi: 10.6004/jnccn.2022.0047

40. Buchanan AH, Datta SK, Skinner CS, Hollowell GP, Beresford HF, Freeland T, et al. Randomized trial of telegenetics vs. in-person cancer genetic counseling: Cost, patient satisfaction and attendance. *J Genet Couns.* (2015) 24(6):961–70. doi: 10.1007/s10897-015-9836-6

41. Schwartz MD, Valdimarsdottir HB, Peshkin BN, Mandelblatt J, Nusbaum R, Huang AT, et al. Randomized noninferiority trial of telephone versus in-person genetic counseling for hereditary breast and ovarian cancer. *J Clin Oncol* (2014) 32(7):618–26. doi: 10.1200/JCO.2013.51.3226

42. Ryan NA, Donnelly L, Stocking K, Evans DG, Crosbie EJ. Feasibility of gynaecologist led lynch syndrome testing in women with endometrial cancer. *J Clin Med* (2020) 9(6):1842. doi: 10.3390/jcm9061842

43. Nazareth S, Nussbaum RL, Siglen E, Wicklund CA. Chatbots & artificial intelligence to scale genetic information delivery. *J Genet Couns.* (2021) 30(1):7–10. doi: 10.1002/jgc4.1359

44. Morgan KM, Hamilton JG, Symecko H, Kamara D, Jenkins C, Lester J, et al. Targeted BRCA1/2 population screening among ashkenazi Jewish individuals using a web-enabled medical model: An observational cohort study. *Genet Med* (2022) 24(3):564–75. doi: 10.1016/j.gim.2021.10.016

45. Frey MK, Finch A, Kulkarni A, Akbari MR, Chapman-Davis E. Genetic testing for all: Overcoming disparities in ovarian cancer genetic testing. *Am Soc Clin Oncol Educ Book* (2022) 42:471–82. doi: 10.1200/EDBK_350292

46. Weiss JM, Gupta S, Burke CA, Axell L, Chen LM, Chung DC, et al. NCCN guidelines® insights: Genetic/Familial high-risk assessment: Colorectal, version 1.2021. *J Natl Compr Canc Netw* (2021) 19(10):1122–32. doi: 10.1164/jnccn.2021.0048

47. *National Comprehensive Cancer Network. Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic* (Version 1.2023) (2023). Available from: https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf

48. Kurian AW, Ward KC, Abrahamse P, Bondarenko I, Hamilton AS, Deapen D, et al. Time trends in receipt of germline genetic testing and results for women diagnosed with breast cancer or ovarian cancer, 2012-2019. *J Clin Oncol* (2021) 39(15):1631–40. doi: 10.1200/JCO.20.02785

49. Esterling L, Wijayatunge R, Brown K, Morris B, Hughes E, Pruss D, et al. Impact of a cancer gene variant reclassification program over a 20-year period. *JCO Precis Oncol* (2020) 4:PO.20.00020. doi: 10.1200/PO.20.00020

50. Hampel H. Genetic counseling and cascade genetic testing in lynch syndrome. *Familial Cancer.* (2016) 15(3):423–7. doi: 10.1007/s10689-016-9893-5

51. Grosse SD. When is genomic testing cost-effective? testing for lynch syndrome in patients with newly-diagnosed colorectal cancer and their relatives. *Healthcare* (2015) 3(4):860–78. doi: 10.3390/healthcare3040860

52. Ryan NA, McMahon RF, Ramchander NC, Seif MW, Evans DG, Crosbie EJ. Lynch syndrome for the gynaecologist. *Obstet Gynaecol.* (2021) 23(1):9–20. doi: 10.1111/tog.12706

53. Renkonen-Sinisalo L, Bützow R, Leminen A, Lehtovirta P, Mecklin JP, Järvinen HJ. Surveillance for endometrial cancer in hereditary nonpolyposis colorectal cancer syndrome. *Int J Cancer.* (2007) 120(4):821–4. doi: 10.1002/ijc.22446

54. Dove-Edwin I, Boks D, Goff S, Kenter GG, Carpenter R, Vasen HF, et al. The outcome of endometrial carcinoma surveillance by ultrasound scan in women at risk of hereditary nonpolyposis colorectal carcinoma and familial colorectal carcinoma. *Cancer* (2002) 94(6):1708–12. doi: 10.1002/cncr.10380

55. Auranen A, Joutsiniemi T. A systematic review of gynecological cancer surveillance in women belonging to hereditary nonpolyposis colorectal cancer (Lynch syndrome) families. *Acta Obstet Gynecol Scand* (2011) 90(5):437–44. doi: 10.1111/j.1600-0412.2011.01091.x

56. Crosbie EJ, Ryan NAJ, Arends MJ, Bosse T, Burn J, Cornes JM, et al. The Manchester international consensus group recommendations for the management of gynecological cancers in lynch syndrome. *Genet Med* (2019) 21(10):2390–400. doi: 10.1038/s41436-019-0489-y

57. Dijkhuizen FP, Mol BW, Brölmann HA, Heintz AP. The accuracy of endometrial sampling in the diagnosis of patients with endometrial carcinoma and hyperplasia: a meta-analysis. *Cancer* (2000) 89(8):1765–72. doi: 10.1002/1097-0142(20001015)89:8<1765::AID-CNCR17>3.0.CO;2-F

58. Nebgen DR, Lu KH, Rimes S, Keeler E, Broaddus R, Munsell MF, et al. Combined colonoscopy and endometrial biopsy cancer screening results in women with lynch syndrome. *Gynecologic Oncol* (2014) 135(1):85–9. doi: 10.1016/j.ygyno.2014.08.017

59. Gerritzen LH, Hoogerbrugge N, Oei AL, Nagengast FM, van Ham MA, Massuger LF, et al. Improvement of endometrial biopsy over transvaginal ultrasound alone for endometrial surveillance in women with lynch syndrome. *Fam Cancer.* (2009) 8(4):391–7. doi: 10.1007/s10689-009-9252-x

60. Zhao S, Chen L, Zang Y, Liu W, Liu S, Teng F, et al. Endometrial cancer in lynch syndrome. *Int J Cancer.* (2022) 150(1):7–17. doi: 10.1002/ijc.33763

61. Bakkum-Gamez JN, Wentzensen N, Maurer MJ, Hawthorne KM, Voss JS, Kroneman TN, et al. Detection of endometrial cancer *via* molecular analysis of DNA collected with vaginal tampons. *Gynecol Oncol* (2015) 137(1):14–22. doi: 10.1016/j.ygyno.2015.01.552

62. Sangtani A, Wang C, Weaver A, Hoppman NL, Kerr SE, Abyzov A, et al. Combining copy number, methylation markers, and mutations as a panel for endometrial cancer detection *via* intravaginal tampon collection. *Gynecol Oncol* (2020) 156(2):387–92. doi: 10.1016/j.ygyno.2019.11.028

63. Wang Y, Li L, Douville C, Cohen JD, Yen TT, Kinde I, et al. Evaluation of liquid from the papanicolaou test and other liquid biopsies for the detection of endometrial and ovarian cancers. *Sci Transl Med* (2018) 10(433). doi: 10.1126/scitranslmed.aap8793

64. Njoku K, Chiasserini D, Jones ER, Barr CE, O'Flynn H, Whetton AD, et al. Urinary biomarkers and their potential for the non-invasive detection of endometrial cancer. *Front Oncol* (2020) 10:559016. doi: 10.3389/fonc.2020.559016

65. Shao X, Wang K, Liu X, Gu C, Zhang P, Xie J, et al. Screening and verifying endometrial carcinoma diagnostic biomarkers based on a urine metabolomic profiling study using UPLC-Q-TOF/MS. *Clin Chim Acta* (2016) 463:200–6. doi: 10.1016/j.cca.2016.10.027

66. Zhao H, Jiang Y, Liu Y, Yun C, Li L. Endogenous estrogen metabolites as biomarkers for endometrial cancer *via* a novel method of liquid chromatography-mass spectrometry with hollow fiber liquid-phase microextraction. *Horm Metab Res* (2015) 47(2):158–64. doi: 10.1055/s-0034-1371865

67. Stoffel EM, Mangu PB, Gruber SB, Hamilton SR, Kalady MF, Lau MW, et al. 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. *J Clin Oncol* (2015) 33(2):209–17. doi: 10.1200/JCO.2014.58.1322

68. Kwon JS, Sun CC, Peterson SK, White KG, Daniels MS, Boyd-Rogers SG, et al. Cost-effectiveness analysis of prevention strategies for gynecologic cancers in lynch syndrome. *Cancer* (2008) 113(2):326–35. doi: 10.1002/cncr.23554

69. Ring KL, Mills AM, Modesitt SC. Endometrial hyperplasia. *Obstetrics Gynecology.* (2022) 140(6):1061–75. doi: 10.1097/AOG.0000000000004989

70. Burn J, Sheth H, Elliott F, Reed L, Macrae F, Mecklin JP, 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(10240):1855–63. doi: 10.1016/S0140-6736(20)30366-4

71. Centers for Disease ControlCombination oral contraceptive use and the risk of endometrial cancer. the cancer and steroid hormone study of the centers for disease control and the national institute of child health and human development. *JAMA* (1987) 257(6):796–800. doi: 10.1001/jama.1987.03390060086029

72. Benshushan A, Paltiel O, Rojansky N, Brzezinski A, Laufer N. IUD Use and the risk of endometrial cancer. *Eur J Obstet Gynecol Reprod Biol* (2002) 105(2):166–9. doi: 10.1016/S0301-2115(02)00153-7

73. Collaborative Group on Epidemiological Studies on Endometrial CancerEndometrial cancer and oral contraceptives: An individual participant meta-analysis of 27 276 women with endometrial cancer from 36 epidemiological studies. *Lancet Oncol* (2015) 16(9):1061–70. doi: 10.1016/S1470-2045(15)00212-0

74. Dumesic DA, Lobo RA. Cancer risk and PCOS. *Steroids* (2013) 78(8):782–5. doi: 10.1016/j.steroids.2013.04.004

75. Dashti SG, Chau R, Ouakrim DA, Buchanan DD, Clendenning M, Young JP, et al. Female hormonal factors and the risk of endometrial cancer in lynch syndrome. *JAMA* (2015) 314(1):61–71. doi: 10.1001/jama.2015.6789

76. Lu KH, Loose DS, Yates MS, Nogueras-Gonzalez GM, Munsell MF, Chen LM, et al. Prospective multicenter randomized intermediate biomarker study of oral contraceptive versus depo-provera for prevention of endometrial cancer in women with lynch syndrome. *Cancer Prev Res (Phila).* (2013) 6(8):774–81. doi: 10.1158/1940-6207.CAPR-13-0020

77. Jareid M, Thalabard J-C, Aarflot M, Bøvelstad HM, Lund E, Braaten T. Levonorgestrel-releasing intrauterine system use is associated with a decreased risk of ovarian and endometrial cancer, without increased risk of breast cancer. results from the NOWAC study. *Gynecologic Oncol* (2018) 149(1):127–32. doi: 10.1016/j.ygyno.2018.02.006

78. Soini T, Hurskainen R, Grénman S, Mäenpää J, Paavonen J, Pukkala E. Cancer risk in women using the levonorgestrel-releasing intrauterine system in Finland. *Obstetrics Gynecology.* (2014) 124(2 PART 1):292–9. doi: 10.1097/AOG.0000000000000356

79. Wan YL, Holland C. The efficacy of levonorgestrel intrauterine systems for endometrial protection: A systematic review. *Climacteric* (2011) 14(6):622–32. doi: 10.3109/13697137.2011.579650

80. Schorge JO, Modesitt SC, Coleman RL, Cohn DE, Kauff ND, Duska LR, et al. SGO white paper on ovarian cancer: Etiology, screening and surveillance. *Gynecol Oncol* (2010) 119(1):7–17. doi: 10.1016/j.ygyno.2010.06.003

81. Hermsen BB, Olivier RI, Verheijen RH, van Beurden M, de Hullu JA, Massuger LF, et al. No efficacy of annual gynaecological screening in BRCA1/2 mutation carriers; an observational follow-up study. *Br J Cancer.* (2007) 96(9):1335–42. doi: 10.1038/sj.bjc.6603725

82. Kobayashi H, Yamada Y, Sado T, Sakata M, Yoshida S, Kawaguchi R, et al. A randomized study of screening for ovarian cancer: a multicenter study in Japan. *Int J Gynecol Cancer.* (2008) 18(3):414–20. doi: 10.1111/j.1525-1438.2007.01035.x

83. Stirling D, Evans DG, Pichert G, Shenton A, Kirk EN, Rimmer S, et al. Screening for familial ovarian cancer: Failure of current protocols to detect ovarian cancer at an early stage according to the international federation of gynecology and obstetrics system. *J Clin Oncol* (2005) 23(24):5588–96. doi: 10.1200/JCO.2005.05.097

84. Buys SS, Partridge E, Black A, Johnson CC, Lamerato L, Isaacs C, et al. Effect of screening on ovarian cancer mortality: The prostate, lung, colorectal and ovarian (PLCO) cancer screening randomized controlled trial. *JAMA* (2011) 305(22):2295–303. doi: 10.1001/jama.2011.766

85. Ryan NAJ, Evans DG, Green K, Crosbie EJ. Pathological features and clinical behavior of lynch syndrome-associated ovarian cancer. *Gynecol Oncol* (2017) 144 (3):491–5. doi: 10.1016/j.ygyno.2017.01.005

86. Oncology SoG. *SGO clinical practice statement: Salpingectomy for ovarian cancer prevention*. IL: SGO Chicago (2013).

87. Chambers SK, Hess LM. Ovarian cancer prevention. *Fundamentals Cancer Prevention: Springer;* (2014), 557–91. doi: 10.1007/978-3-642-38983-2_18

88. Liu Q, Simin J, Debelius J, Fall K, Sadr-Azodi O, Engstrand L, et al. Menopausal hormone therapies and risk of colorectal cancer: A Swedish matched-cohort study. *Aliment Pharmacol Ther* (2021) 53(11):1216–25. doi: 10.1111/apt.16362

89. Lessard-Anderson CR, Handlogten KS, Molitor RJ, Dowdy SC, Cliby WA, Weaver AL, et al. Effect of tubal sterilization technique on risk of serous epithelial ovarian and primary peritoneal carcinoma. *Gynecologic Oncol* (2014) 135(3):423–7. doi: 10.1016/j.ygyno.2014.10.005

90. Madsen C, Baandrup L, Dehlendorff C, Kjær SK. Tubal ligation and salpingectomy and the risk of epithelial ovarian cancer and borderline ovarian tumors: A nationwide case–control study. *Acta Obstetricia Gynecologica Scandinavica.* (2015) 94(1):86–94. doi: 10.1111/aogs.12516

91. Falconer H, Yin L, Grönberg H, Altman D. Ovarian cancer risk after salpingectomy: A nationwide population-based study. *JNCI: J Natl Cancer Institute.* (2015) 107(2). doi: 10.1093/jnci/dju410

92. Nakamura K, Banno K, Yanokura M, Iida M, Adachi M, Masuda K, et al. Features of ovarian cancer in lynch syndrome (Review). *Mol Clin Oncol* (2014) 2 (6):909–16. doi: 10.3892/mco.2014.397

93. Swanson CL, Bakkum-Gamez JN. Preventing ovarian cancer in high-risk women: One surgery at a time. *Clin Obstet Gynecol.* (2020) 63(1):64–73. doi: 10.1097/GRF.0000000000000499

94. Centers for Disease ControlOral contraceptive use and the risk of ovarian cancer. the centers for disease control cancer and steroid hormone study. *JAMA* (1983) 249(12):1596–9. doi: 10.1001/jama.1983.03330360036032

95. Havrilesky LJ, Moorman PG, Lowery WJ, Gierisch JM, Coeytaux RR, Urrutia RP, et al. Oral contraceptive pills as primary prevention for ovarian cancer: A systematic review and meta-analysis. *Obstet Gynecol.* (2013) 122(1):139–47. doi: 10.1097/AOG.0b013e318291c235

96. Iodice S, Barile M, Rotmensz N, Feroce I, Bonanni B, Radice P, et al. Oral contraceptive use and breast or ovarian cancer risk in BRCA1/2 carriers: A meta-analysis. *Eur J Cancer.* (2010) 46(12):2275–84. doi: 10.1016/j.ejca.2010.04.018

97. Schrijver LH, Antoniou AC, Olsson H, Mooij TM, Roos-Blom MJ, Azarang L, et al. Oral contraceptive use and ovarian cancer risk for BRCA1/2 mutation carriers: An international cohort study. *Am J Obstet Gynecol.* (2021) 225(1):51.e1–.e17. doi: 10.1016/j.ajog.2021.01.014

98. Roudko V, Cimen Bozkus C, Greenbaum B, Lucas A, Samstein R, Bhardwaj N. Lynch syndrome and MSI-h cancers: From mechanisms to "Off-The-Shelf" cancer vaccines. *Front Immunol* (2021) 12:757804. doi: 10.3389/fimmu.2021.757804

99. ClinicalTrials.gov: U.S. National Library of Medicine. Available at: https://clinicaltrials.gov.

100. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* (2015) 372 (26):2509–20. doi: 10.1056/NEJMoa1500596

101. Kim SR, Pina A, Albert A, McAlpine J, Wolber R, Blake Gilks C, et al. Does MMR status in endometrial cancer influence response to adjuvant therapy? *Gynecol Oncol* (2018) 151(1):76–81. doi: 10.1016/j.ygyno.2018.08.020

102. Reijnen C, Küsters-Vandevelde HVN, Prinsen CF, Massuger L, Snijders M, Kommoss S, et al. Mismatch repair deficiency as a predictive marker for response to adjuvant radiotherapy in endometrial cancer. *Gynecol Oncol* (2019) 154(1):124–30. doi: 10.1016/j.ygyno.2019.03.097

103. Li JY, Park HS, Huang GS, Young MR, Ratner E, Santin A, et al. Prognostic impact of mismatch repair deficiency in high- and low-intermediate-risk, early-stage endometrial cancer following vaginal brachytherapy. *Gynecol Oncol* (2021) 163(3):557–62. doi: 10.1016/j.ygyno.2021.09.018

104. León-Castillo A, Boer S, Powell ME, Mileshkin LR, Mackay HJ, Leary A, et al. Molecular classification of the PORTEC-3 trial for high-risk endometrial cancer: Impact on prognosis and benefit from adjuvant therapy. *J Clin Oncol* (2020) 38 (29):3388–97. doi: 10.1200/JCO.20.00549

105. RAINBO Research Consortium. Refining adjuvant treatment in endometrial cancer based on molecular features: the RAINBO clinical trial program. *Int J Gynecol Cancer.* (2022) 33(1):109–17. doi: 10.1136/ijgc-2022-004039

106. Hou X. *Study of early stage endometrial cancer based on molecular classification and traditional risk stratification to guide adjuvant radiotherapy decisions* (2022). Available at: https://clinicaltrials.gov.

107. van den Heerik A, Horeweg N, Nout RA, Lutgens L, van der Steen-Banasik EM, Westerveld GH, et al. PORTEC-4a: International randomized trial of molecular profile-based adjuvant treatment for women with high-intermediate risk endometrial cancer. *Int J Gynecol Cancer.* (2020) 30(12):2002–7. doi: 10.1136/ijgc-2020-001929

108. Rombouts AJM, Hugen N, Elferink MAG, Poortmans PMP, Nagtegaal ID, de Wilt JHW. Increased risk for second primary rectal cancer after pelvic radiation therapy. *Eur J Cancer.* (2020) 124:142–51. doi: 10.1016/j.ejca.2019.10.022

109. Wiltink LM, Nout RA, Fiocco M, Meershoek-Klein Kranenbarg E, Jürgenliemk-Schulz IM, Jobsen JJ, et al. No increased risk of second cancer after radiotherapy in patients treated for rectal or endometrial cancer in the randomized TME, PORTEC-1, and PORTEC-2 trials. *J Clin Oncol* (2015) 33(15):1640–6. doi: 10.1200/JCO.2014.58.6693

110. Xiao X, Melton DW, Gourley C. Mismatch repair deficiency in ovarian cancer – molecular characteristics and clinical implications. *Gynecol Oncol* (2014) 132(2):506–12. doi: 10.1016/j.ygyno.2013.12.003

111. Scartozzi M, De Nictolis M, Galizia E, Carassai P, Bianchi F, Berardi R, et al. Loss of hMLH1 expression correlates with improved survival in stage III-IV ovarian cancer patients. *Eur J Cancer.* (2003) 39(8):1144–9. doi: 10.1016/S0959-8049(03)00197-7

112. Grindedal EM, Renkonen-Sinisalo L, Vasen H, Evans G, Sala P, Blanco I, et al. Survival in women with MMR mutations and ovarian cancer: A multicentre study in lynch syndrome kindreds. *J Med Genet* (2010) 47(2):99–102. doi: 10.1136/jmg.2009.068130

113. Zhao C, Li S, Zhao M, Zhu H, Zhu X. Prognostic values of DNA mismatch repair genes in ovarian cancer patients treated with platinum-based chemotherapy. *Arch Gynecol Obstet.* (2018) 297(1):153–9. doi: 10.1007/s00404-017-4563-x

114. Marcus L, Lemery SJ, Keegan P, Pazdur R. FDA Approval summary: Pembrolizumab for the treatment of microsatellite instability-high solid tumors. *Clin Cancer Res* (2019) 25(13):3753–8. doi: 10.1158/1078-0432.CCR-18-4070

115. Deshpande M, Romanski PA, Rosenwaks Z, Gerhardt J. Gynecological cancers caused by deficient mismatch repair and microsatellite instability. *Cancers (Basel).* (2020) 12(11):3319. doi: 10.3390/cancers12113319

116. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite Instability/Mismatch repair-deficient cancer: Results from the phase II KEYNOTE-158 study. *J Clin Oncol* (2020) 38(1):1–10. doi: 10.1200/JCO.19.02105

117. Slomovitz BM, Cibula D, Simsek T, Mirza MR, Maćkowiak-Matejczk B, Hudson E, et al. KEYNOTE-C93/GOG-3064/ENGOT-en15: A phase 3, randomized, open-label study of first-line pembrolizumab versus platinum-doublet chemotherapy in mismatch repair deficient advanced or recurrent endometrial carcinoma. *J Clin Oncol* (2022) 40(16_suppl):TPS5623–TPS. doi: 10.1200/JCO.2022.40.16_suppl.TPS5623

118. *Endometrial cancer patients MMR deficient comparing chemotherapy vs dostarlimab in first line (DOMENICA)* . Available at: https://clinicaltrials.gov.

119. Coutzac C, Bibeau F, Ben Abdelghani M, Aparicio T, Cohen R, Coquan E, et al. Immunotherapy in MSI/dMMR tumors in the perioperative setting: The IMHOTEP trial. *Dig Liver Dis* (2022) 54(10):1335–41. doi: 10.1016/j.dld.2022.07.008

120. Trojan J, Stintzing S, Haase O, Koch C, Ziegler P, Demes M, et al. Complete pathological response after neoadjuvant short-course immunotherapy with ipilimumab and nivolumab in locally advanced MSI-H/dMMR rectal cancer. *Oncologist* (2021) 26 (12):e2110–e4. doi: 10.1002/onco.13955

121. Kinney RE, Khalil M. Neoadjuvant immunotherapy in microsatellite-instability high nonmetastatic colorectal cancer: A single-institute experience and review of the literature. *Clin Colorectal Cancer.* (2021) 20(2):e109–e12. doi: 10.1016/j.clcc.2020.10.002

122. Demisse R, Damle N, Kim E, Gong J, Fakih M, Eng C, et al. Neoadjuvant immunotherapy-based systemic treatment in MMR-deficient or MSI-high rectal cancer: Case series. *J Natl Compr Canc Netw* (2020) 18(7):798–804. doi: 10.6004/jnccn.2020.7558

frontiers | Frontiers in Oncology

TYPE Case Report
PUBLISHED 03 March 2023
DOI 10.3389/fonc.2023.1126476

Check for updates

OPEN ACCESS

EDITED BY
Toni T. Seppälä,
Helsinki University Central Hospital, Finland

REVIEWED BY
Jussi Nikkola,
Tampere University Hospital, Finland
Teemu Murtola,
Tampere University, Finland

*CORRESPONDENCE
Danielle M. Pastor
danielle.pastor@nih.gov

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 17 December 2022
ACCEPTED 16 February 2023
PUBLISHED 03 March 2023

CITATION
Atiq MO, Pastor DM, Karzai F, Hankin AR, Turkbey B, Cordes LM, Brownell I, Liu Y, Chesnut GT and Madan RA (2023) First-line pembrolizumab plus androgen deprivation therapy for locally advanced microsatellite instability-high prostate cancer in a patient with Muir-Torre syndrome: A case report. *Front. Oncol.* 13:1126476. doi: 10.3389/fonc.2023.1126476

COPYRIGHT
© 2023 Atiq, Pastor, Karzai, Hankin, Turkbey, Cordes, Brownell, Liu, Chesnut and Madan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

First-line pembrolizumab plus androgen deprivation therapy for locally advanced microsatellite instability-high prostate cancer in a patient with Muir-Torre syndrome: A case report

Mohammad O. Atiq[1†], Danielle M. Pastor[2*†], Fatima Karzai[1], Amy R. Hankin[1], Baris Turkbey[3], Lisa M. Cordes[2], Isaac Brownell[2,4], Yi Liu[5], Gregory T. Chesnut[6,7] and Ravi A. Madan[1]

[1]Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States, [2]Center for Immuno-Oncology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States, [3]Molecular Imaging Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States, [4]Dermatology Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD, United States, [5]Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States, [6]Urology Service, Department of Surgery, Walter Reed National Military Medical Center, Bethesda, MD, United States, [7]Center for Prostate Disease Research, Murtha Cancer Center Research Program, Department of Surgery, Uniformed Services University of the Health Sciences, Bethesda, MD, United States

The risks of development of colorectal and endometrial cancers in individuals with Lynch syndrome (LS) are well known and have been widely studied. In recent years, the potential association of other malignancies, including prostate cancer, with LS has been considered. Decision-making regarding screening for prostate cancer in the generalized population can be complicated; accounting for the possibility of a higher risk of cancer conferred by a potential genetic predisposition confounds the creation of salient guidelines even further. Although tissue-agnostic treatment approvals have been granted to several immune checkpoint inhibitors (ICIs) for their use in the treatment of subsets of patients whose tumors exhibit high levels of microsatellite instability or high tumor mutational burden, a paucity of data exists regarding the use of ICIs in the first line treatment of patients with locally advanced prostate cancer harboring these features. A significant reduction in tumor volume in response to the combination of immune checkpoint inhibition and androgen deprivation therapy is described in this report of a male with Muir-Torre syndrome who was found to have locally advanced adenocarcinoma of the prostate. While

anecdotal, the anti-tumor activity of this combination of therapy is notable and calls attention to the importance of considering further investigation of the use of immune checkpoint blockade as a primary therapeutic option in patients with localized prostate cancer.

KEYWORDS

Muir-Torre Syndrome, lynch syndrome, prostate cancer, immunotherapy, microsatellite instability (MSI), mismatch repair genes, immune checkpoint inhibitor, androgen deprivation therapy (ADT)

Introduction

Pembrolizumab is a programmed cell death-1 (PD-1) receptor blocking antibody that is authorized for use by the U.S. Food and Drug Administration for a number of indications in the treatment of solid tumor malignancies, including use as monotherapy, in combination with chemotherapeutic agents, and in conjunction with targeted therapy (1). Its role as a therapeutic option for individuals with prostate cancer is limited, however, and extends only to the small subset of patients whose tumors exhibit high levels of microsatellite instability (MSI-H), are deficient in mismatch repair (dMMR), and/or express high tumor mutational burden (TMB-H). Lynch syndrome (LS) refers to an autosomal dominant disorder associated with inactivating germline mutations in DNA MMR genes or structural variants at the *EPCAM* locus silencing MSH2 protein expression. Individuals with pathogenic germline variants of these MMR gene mutations are predisposed to the development of multiple types of cancers throughout their lifetime (2). Malignancies that develop in this setting typically exhibit high levels of MSI, secondary to impaired gene replication and disrupted DNA homeostasis which lead to genetic hypermutability and changes in microsatellite length (3). In recent years, prostate cancer has increasingly been suggested as a tumor type associated with this syndrome (4–7). Although literature exists regarding the use of pembrolizumab in patients with LS and metastatic prostate cancer, to our knowledge, there have not been any reports to date describing the use of pembrolizumab in the first-line setting for LS patients with locally advanced prostate cancer. In this report, we describe a patient with locally advanced, MSI-H prostate cancer in the setting of Muir-Torre syndrome (MTS), a rare phenotypic variant of LS associated with sebaceous carcinoma risk, who has experienced a robust response to first-line treatment with the combination of pembrolizumab and androgen deprivation therapy (ADT). Although dMMR/MSI-H cancers of the prostate are, overall, uncommon, the impact of this molecular phenotype on clinical decision-making for those patients with tumors harboring these features is significant.

Case description

A 70-year-old man developed lower urinary tract symptoms. His past medical history was notable for a low-grade urothelial carcinoma of the bladder (treated with transurethral resection of bladder tumor and intravesical BCG), right-sided colon cancer (treated with hemicolectomy and systemic 5-fluorouracil), squamous cell carcinoma of the tonsil (treated with resection and systemic cisplatin, followed by chemoradiation with 5-fluorouracil as a radiosensitizing agent), and numerous cutaneous squamous cell carcinomas and sebaceous carcinomas. A strong family history of cancer was evident and included a daughter with metastatic ovarian cancer in the setting of MTS, a father with brain and duodenal cancers, and a half-sister with MTS. Family history was also notable for multiple siblings with various malignancies, including cervical, colon, and oral cancer of unknown type.

Shortly after the onset of his urinary symptoms, the patient developed intermittent bowel incontinence and gross hematuria. He presented for evaluation and a transurethral resection of the prostate was recommended. He ultimately underwent transurethral resections of the prostate, bladder neck, and prostatic urethra, all specimens of which were confirmed to be prostatic adenocarcinoma with features consistent with very high risk prostate cancer (Gleason 5 + 4, with 95% grade group 5, with >80% involvement of tumor within resected tissue and extraprostatic extension [EPE] into the lamina propria of the urothelium). Positive expression of both prostate-specific antigen (PSA) and NKX3.1 in all specimens was determined by immunohistochemical (IHC) evaluation. Serum PSA level was found to be 12.36 ng/mL. Magnetic resonance imaging (MRI) of the pelvis that was performed after transurethral resection revealed circumferential involvement of the rectum with extension into the base of the penis with no lymphadenopathy (Figure 1). No visceral nor bone metastases were demonstrated on computed tomography scan nor bone scintigraphy, respectively. The patient then underwent a colonoscopy with biopsy of rectal mass, as well as repeat biopsies of the bladder trigone and prostatic urethra; all specimens exhibited positive expression of PSA, NKX3.1, and CDX2 by IHC evaluation, further supporting the diagnosis of invasive prostatic adenocarcinoma. Additional analysis of the original prostate pathology specimen revealed loss of nuclear positivity of MSH2 and MSH6 with intact nuclear positivity of MLH1 and PMS2. Microsatellite testing by PCR revealed the specimen to be MSI-H. Prior germline genetic testing had established that the patient carried a pathogenic variant in c.319delinsAATAAGGCATC

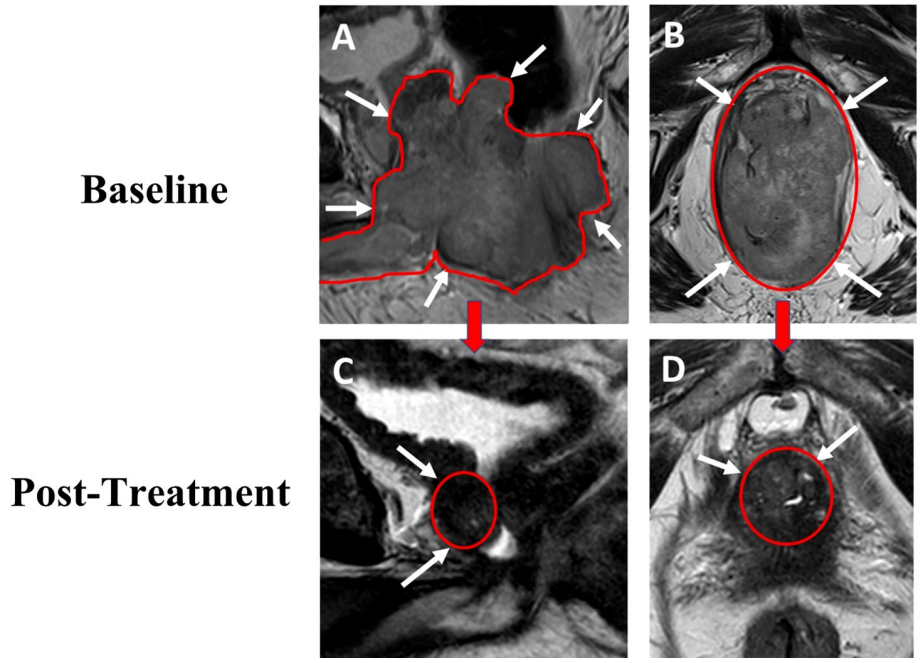


FIGURE 1
69-year-old male with advanced prostate cancer. Baseline sagittal **(A)** and axial **(B)** T2W MRI performed after transurethral resection show a large mass (total lesion volume of 282cc) occupying the whole prostate with rectum, bladder with diffusion restriction on ADC map. Post-treatment follow up sagittal **(C)** and axial **(D)** T2W MRI show a gradual decrease in size of the lesion (total lesion volume 11.4cc) without evidence of rectum and bladder involvement. T2W, T2 weighted image; ADC, apparent diffusion coefficient.

(p.Ala107fs) in the *MSH2* gene and a variant of uncertain significance c.1816C>T (p.Pro606Ser) in the *BRCA2* gene.

One month following his transurethral resections, the patient developed new symptoms of coccydynia, constipation, bowel incontinence, and change in stool caliber. Serum PSA level at this time was 10.92 ng/mL. The extent of local disease and his other comorbidities, including congestive heart failure, atrial fibrillation, and asthma, prompted physicians to seek potential alternative initial treatment strategies to radiation treatment or operative intervention, particularly given the molecular characteristic of MSI-H disease. He was evaluated at the National Cancer Institute of the National Institutes of Health and, in consideration of the dMMR and MSI-H status of his tumor, treatment was initiated with pembrolizumab, in combination with ADT without concomitant androgen blockers. Treatment was comprised of pembrolizumab 200 mg administered intravenously every 3 weeks, with degarelix 240 mg loading dose given as 2 subcutaneous injections followed by a singular subcutaneous 80 mg maintenance dose given every 4 weeks, prior to eventually transitioning to leuprolide acetate for depot suspension 22.5 mg given as a single intramuscular injection every 12 weeks. Within 24 hours of initial pembrolizumab infusion, the patient reported near-complete resolution of straining with defecation, with marked improvement of urinary symptoms. Within one month of initiation of therapy, his serum PSA level was undetectable (Figure 2). Multiparametric MRI of the prostate was obtained after 2 cycles of treatment with pembrolizumab plus ADT and showed a decrease in size in the intraprostatic lesion, from 8.9 cm to 2.8 cm (Figure 1). This radiographic finding corresponded to a reduction in prostate volume from an original total lesion volume of 282 cc to a post-treatment volume of 11.4 cc. Extraprostatic extension was evident posteriorly and laterally, to the right of the prostate; invasion of the seminal vesicles was visible at the root. Notably, the tumor involvement of the bladder and rectum that had been previously identified was no longer visualized. The patient was continued on pembrolizumab plus ADT. A subsequent MRI of the prostate was performed at six months of therapy and demonstrated a further decrease in the greatest dimension of the intraprostatic lesion at 2.1 cm. Additionally, EPE had resolved per imaging.

During his treatment, the patient developed verrucous cutaneous lesions. Dermatology was consulted and a biopsy was performed which confirmed benign lichenoid keratoses, a known dermatologic effect of pembrolizumab. The patient's PSA level has remained undetectable at 19.5 months since initiation of treatment and continuation of pembrolizumab plus ADT is planned for a total of 2 years, with serial monitoring of his PSA level and repeated MRI of the prostate to be performed at scheduled intervals.

Discussion

Lynch syndrome is an inherited disorder known to confer an increased lifetime risk of the development of several types of cancers to those individuals harboring pathognomonic germline mutations of genes associated with dysfunctional DNA MMR. Although the associations of colorectal and endometrial malignancies with this syndrome have long been well-established, estimated risks of other tumor types have also been demonstrated to be higher than those

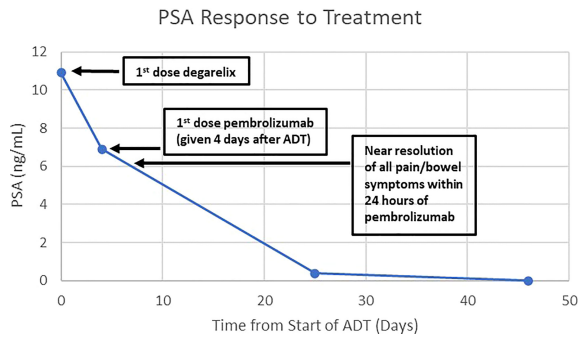


FIGURE 2
Graph of the patient's PSA response to treatment with degarelix and pembrolizumab. The patient was treated with degarelix (denoted at Day 0) when his PSA was 10.92 ng/mL. Four days later, he was given pembrolizumab when his PSA was 6.9 ng/mL. His symptoms from the prostate mass largely resolved within 24 hours of the first dose of pembrolizumab. The PSA became undetectable at day 46 from start of degarelix.

posed to the general, unaffected population. Gastric, intestinal, hepatobiliary, pancreatic, and epithelial ovarian cancers have been accepted as constituent malignancies of the syndrome, as have certain genitourinary malignancies, of which renal pelvic, ureteral, and bladder have been recognized. In recent years, however, prostate cancer has also been proposed to be linked with LS (7, 8).

The increased risk of cancer development in LS is attributed to the presence of germline mutations in DNA MMR genes (*MLH1, MSH2, MSH6, PMS2, EPCAM)*), which are propagated through an autosomal dominant pattern of inheritance (9). The majority of resultant tumors exhibit MSI, a tumor characteristic now recognized to be associated with immunotherapy responsivity (10, 11). Similarly, tumors exhibiting MSI caused by sporadic, acquired hypermethylation of the promoter of the *MLH1* gene also seem to demonstrate improved responses to immune-based therapies (12, 13).

The recognition of the relevance of somatic mutations of DNA repair pathway genes in individuals with prostate cancer to treatment response has not only impacted therapy algorithms but has also influenced recommendations and guidelines regarding germline testing in this patient population (14–17). Multiple studies have demonstrated that proportions of individuals with prostate tumors expressing somatic mutations in either homologous recombination DNA repair pathway genes or genes governing mismatch base excision repair also carry related germline mutations (18–21). These discoveries have led to increasing efforts to identify the risk of prostate cancer development in individuals with LS. Through the evaluation of more than 15,000 various types of tumors, Latham et al. have suggested that MSI/dMMR may be predictive of LS across a more extensive range of cancer type than traditionally appreciated, with 5% of 1048 patients with prostate cancer determined to exhibit MSI-H or MSI-indeterminate (MSI-I) tumors, of which 5.6% were found to have LS (10). In a similar effort to determine the prevalence of MSI in prostate cancer, other investigators found that 3.1% of examined prostate tumors exhibited MSI-H or dMMR; of the patients diagnosed with these tumors, 21.9% carried a pathogenic germline mutation in a LS-associated gene, with mutations in *MSH2* most frequently expressed (4).

In an international prospective, targeted prostate cancer screening study in men aged 40-69 years of age considered to be at genetically higher risk of developing prostate cancer than age-matched controls, more than 600 of 828 males from LS families were found to have germline pathogenic variants in *MLH1*, *MSH2*, and *MSH6* genes (5). These individuals were to undergo annual PSA screening for a minimum of 5 years, with transrectal, ultrasound-guided prostate biopsy recommended for PSA concentration of higher than 3.0 ng/mL. Researchers diagnosed prostate cancer in 4.3% of 305 men with *MSH2* mutations and 3% of 135 men with *MSH6* mutations; conversely, only one of 210 (0.5%) non-carriers in the *MSH2* control group and none of the 177 non-carriers in the *MSH6* control group were found to have prostate cancer. Individuals with *MSH2* mutations were eight times more likely to be diagnosed than their non-carrier counterpart and were diagnosed at younger ages (an average of 58 years versus 66 years, respectively). Further, males with *MSH2* mutations diagnosed with prostate cancer were found to have more aggressive disease than matched control. Patients harboring *MSH6* mutations with prostate cancer were diagnosed at an average age of 62 years; 75% of these individuals were determined to have aggressive disease. Of note, 9 individuals with *MSH2* gene mutations found to have PSA levels greater than 3 ng/mL did not proceed to biopsy, while 5 were found to have benign tissue on biopsy; of the *MSH2* non-carrier controls, 4 who met PSA criteria did not undergo biopsy, while benign tissue was found on biopsies of 2 individuals (5).

The findings in the above studies suggest increased risk for the development of prostate cancer in individuals with LS and underscore the importance of early screening in this population particularly as no consensus currently exists regarding screening in this setting. Currently, prostate cancer screening is not recommended for the general population; rather, the National Comprehensive Cancer Network (NCCN) guidelines suggest that the decision to test baseline PSA level follows an informative discussion between physicians and healthy patients between the ages of 40 - 75 years who are at higher than average risk, such as those with strong family history of prostate cancer, who carry germline mutations that may increase the risk of prostate cancer, and/or with African ancestry (22).

The clinical course of the patient described in this report was further complicated by the presence of MTS. Rarely, LS may manifest with or involve a dermatologic phenotype, with the development of sebaceous adenomas, epitheliomas or carcinomas, and/or keratoacanthomas. Muir-Torre syndrome is a rare variant of LS, more commonly diagnosed in men, with individuals characteristically developing at least one cutaneous tumor and at least one visceral neoplasm (most commonly of gastrointestinal origin, with genitourinary malignancies occurring with second most common frequency) (23). Germline mutations in *MLH1*, *MSH2*, and *MSH6* genes have been implicated in MTS, with *MSH2* mutation occurring most frequently, and both *MLH1* and *MSH2* mutations being associated with more aggressive phenotypes (24, 25).

Treatment of prostate cancer with immune checkpoint blocking monotherapy has been largely unsuccessful, except in a small subset of patients. Two immunotherapies, pembrolizumab and sipuleucel-T, carry indications for their use in the metastatic setting. However, these indications are specific to metastatic castrate-resistant prostate cancer (mCRPC) patients whose tumors are MSI-H/dMMR/TMB-H or to those who have no visceral involvement, respectively. Anti-tumor activity attributed to pembrolizumab has been described in patients with LS with metastatic MSI-H/dMMR prostatic cancer (4, 26). However, as previously mentioned, no reports of checkpoint inhibition as first-line treatment of locally advanced prostate-cancer have been described to our knowledge.

The reduction in the patient's tumor volume resulting from the combination of treatment with pembrolizumab and ADT is more robust than the response expected from treatment with ADT alone. A study of neoadjuvant hormonal therapy given prior to radiation showed that patients administered goserelin for an average of 192 days had a mean prostate volume reduction of 26% (27). The addition of androgen receptor inhibition has been shown to reduce prostate volume to a similar degree. A randomized study examining the resultant prostate volume of patients with localized prostate cancer treated with an average of 3 months of neoadjuvant bicalutamide monotherapy versus bicalutamide plus ADT prior to radiation showed that the mean volume reduction in the monotherapy arm was 17.5%, compared to 28% for the combined therapy arm (28). In a comparison of degarelix versus goserelin, with each administered over 3 months in the neoadjuvant setting, the mean percentage reduction in prostate volume was demonstrated to be -36.0% ± 14.5% versus -35.3% ± 16.7%, respectively (29). These results suggest that an appropriate anticipated estimate for ADT-induced tumor volume reduction might be between 20-40% over 3 months (or, in less stringent terms, a maximal expectation of 50% reduction in tumor volume). Our patient's reduction in tumor volume at 2 months from initiation of ADT was 95.96%. This magnitude of volume reduction is substantially greater than that expected from ADT alone, suggesting that the afforded benefit may essentially be derived from the addition of anti-PD-1 therapy. This also raises the possibility to revisit radiation therapy now that his locally advanced disease appears more localized.

It is uncertain if ADT was essential for the extent of response demonstrated in the individual described herein. Existing data support that ADT can enhance $CD8^+$ T cell infiltration in the prostate tumor microenvironment (30–34). However, a descriptive study conducted by Sommer et al. could not confirm increases in PD-L1 expression after ADT (34). Furthermore, a phase 3 study of patients who would have recently started an ADT-based regimen for newly diagnosed metastatic castration sensitive disease failed to demonstrate that pembrolizumab could improve clinical outcomes over ADT-based therapy, adding to the list of failed PD-1/PD-L1 inhibition trials in prostate cancer (35). Further investigations are required to determine the role that ADT may play in enhancing immunotherapy efficacy in patients with certain genetic mutations such as this patient and if outcomes would be different than in unselected populations.

Recently published data from clinical trials evaluating neoadjuvant immune checkpoint blockade in the treatment of non-metastatic dMMR colon and rectal cancers have illuminated the impact of the "immunoablative" effect of these agents on the potential obviation of additional modalities such as chemoradiotherapy and surgery (36, 37). A prospective phase II study in which single agent dostarlimab, an anti-PD-1 monoclonal antibody, was administered to patients with dMMR locally advanced rectal cancer for six months as neoadjuvant therapy has resulted in striking findings (36). Investigators observed that all 12 patients who had completed treatment and had undergone at least 6 months of follow-up demonstrated clinical complete response, with no evidence of tumor on magnetic resonance imaging, ^{18}F-fluorodeoxyglucose–positron-emission tomography, endoscopic evaluation, digital rectal examination, or biopsy. Patients who achieved clinical complete response were eligible for omission of chemoradiation and surgery; at the time of publication of results, no patients had required chemoradiotherapy or surgery, nor had any cases of disease progression or recurrence been reported during a follow-up period ranging from 6 - 25 months. In the NICHE-2 study, patients with non-metastatic dMMR colon cancer were treated with one dose of ipilimumab and two doses of nivolumab and underwent surgery ≤ 6 weeks of registration (37). With a median time from first dose to surgery of 5 weeks, pathologic response (defined as ≤50% residual viable tumor) was observed in 106/107 (99%) patients, with 102/107 (95%) exhibiting a major pathologic response (defined as ≤10% residual viable tumor), including 67% demonstrating a complete pathologic response. At a median follow-up of 13 months, none of the patients had developed recurrent disease. While longer follow-up is warranted to assess duration of response in these studies, these findings support the movement towards a potential paradigm shift through which immunotherapy could be used at earlier stages of disease in effort to maximize organ-sparing approaches as treatment strategies. Similarly, the possibility that treatment of dMMR/MSI-H locally advanced prostate cancer with immunotherapy could eliminate the need for radical surgery is intriguing, given the emerging data described regarding the use of ICIs in locally advanced dMMR CRC.

Conclusion

The role of checkpoint inhibition in the treatment of prostate cancer remains limited to a select subpopulation of patients. While currently approved indications and published studies relate to mCRPC, there exists potential for utilizing immunotherapeutic agents earlier in the disease process for patients with genetic aberrations such as those seen in LS. By utilizing pembrolizumab in the neoadjuvant setting, physicians may be able to exploit the aforementioned underlying tumor biology in LS patients to yield an augmented reduction in tumor burden as compared to that associated with ADT monotherapy. Further, it is tantalizing to understand how much tumor the patient described still has and/or how long his disease control could continue from the treatment of ADT and pembrolizumab. It also remains unclear if ADT was required for this response or if the patient needs to remain on ADT indefinitely and what his duration of therapy with ICI should be. Subgroups of patients such as those with dMMR/MSI-H locally

advanced disease who may not be considered candidates for local treatment and whose tumors exhibit particular molecular features may experience greater benefit from immune checkpoint inhibition than that expected with ADT alone. Future studies in populations with dMMR/MSI-H, such as individuals with LS, should be considered to evaluate the clinical benefit of immune checkpoint inhibition in the setting of locally advanced prostate cancer.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

MA and DP contributed equally to this work and share first authorship. Conception/Design: MA, DP, FK, RM; Provision of study material or patients: MA, DP, FK, AH, BT, LC, IB, YL, GC, RM; Collection and/or assembly of data: MA, DP, AH, BT; GC; Data analysis and interpretation: BT, IB, YL, FK, RM; Manuscript writing and/or editing: MA, DP, FK, BT, LC, IB, RM; Final approval of manuscript: MA, DP, FK, RM. All authors were responsible for the contribution of important intellectual content. All authors contributed to the article and approved the submitted version.

Acknowledgments

The authors wish to acknowledge support from the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research and the United States Department of Health and Human Services.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. KEYTRUDA® (pembrolizumab). *Prescribing information*. Whitehouse Station, NJ: Merck Sharp & Dohme Corp, a subsidiary of Merck & Co, Inc (2021).

2. Lynch HT, Lanspa S, Shaw T, Casey MJ, Rendell M, Stacey M, et al. Phenotypic and genotypic heterogeneity of lynch syndrome: A complex diagnostic challenge. *Fam Cancer* (2018) 17(3):403–14. doi: 10.1007/s10689-017-0053-3

3. Olave MC, Graham RP. Mismatch repair deficiency: The what, how and why it is important. *Genes Chromosomes Cancer* (2022) 61(6):314–21. doi: 10.1002/gcc.23015

4. Abida W, Cheng ML, Armenia J, Middha S, Autio KA, Vargas HA, et al. Analysis of the prevalence of microsatellite instability in prostate cancer and response to immune checkpoint blockade. *JAMA Oncol* (2019) 5(4):471–8. doi: 10.1001/jamaoncol.2018.5801

5. Bancroft EK, Page EC, Brook MN, Thomas S, Taylor N, Pope J, et al. A prospective prostate cancer screening programme for men with pathogenic variants in mismatch repair genes (IMPACT): Initial results from an international prospective study. *Lancet Oncol* (2021) 22(11):1618–31. doi: 10.1016/S1470-2045(21)00522-2

6. Brandão A, Paulo P, Teixeira MR. Hereditary predisposition to prostate cancer: From genetics to clinical implications. *Int J Mol Sci* (2020) 21(14):5036. doi: 10.3390/ijms21145036

7. Haraldsdottir S, Hampel H, Wei L, Wu C, Frankel W, Bekaii-Saab T, et al. Prostate cancer incidence in males with lynch syndrome. *Genet Med* (2014) 16:553–7. doi: 10.1038/gim.2013.193

8. Raymond VM, Mukherjee B, Wang F, Huang SC, Stoffel EM, Kastrinos F, et al. Elevated risk of prostate cancer among men with lynch syndrome. *J Clin Oncol* (2013) 31(14):1713–8. doi: 10.1200/JCO.2012.44.1238

9. Silva FC, Valentin MD, Ferreira F de O, Carraro DM, Rossi BM. Mismatch repair genes in lynch syndrome: A review. *Sao Paulo Med J* (2009) 127(1):46–51. doi: 10.1590/s1516-31802009000100010

10. Latham A, Srinivasan P, Kemel Y, Shia J, Bandlamudi C, Mandelker D, et al. Microsatellite instability is associated with the presence of lynch syndrome pan-cancer. *J Clin Oncol* (2019) 37(4):286–95. doi: 10.1200/JCO.18.00283

11. Pastor DM, Schlom J. Immunology of lynch syndrome. *Curr Oncol Rep* (2021) 23(8):96. doi: 10.1007/s11912-021-01085-z

12. Le DT, Kim TW, Van Cutsem E, Geva R, Jager D, Hara H, et al. Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: Keynote-164. *J Clin Oncol* (2020) 38(1):11–9. doi: 10.1200/JCO.19.02107

13. Overman MJ, Lonardi S, Wong KYM, Lenz HJ, Gelsomino F, Aglietta M, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J Clin Oncol* (2018) 36(8):773–9. doi: 10.1200/JCO.2017.76.9901

14. Cheng HH, Sokolova AO, Schaeffer EM, Small EJ, Higano CS. Germline and somatic mutations in prostate cancer for the clinician. *J Natl Compr Canc Netw* (2019) 17(5):515–21. doi: 10.6004/jnccn.2019.7307

15. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* (2005) 434(7035):917–21. doi: 10.1038/nature03445

16. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite Instability/Mismatch repair-deficient cancer: Results from the phase II KEYNOTE-158 study. *J Clin Oncol* (2020) 38(1):1–10. doi: 10.1200/JCO.19.02105

17. Mateo J, Porta N, Bianchini D, McGovern U, Elliott T, Jones R, et al. Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPARP-b): A multicentre, open-label, randomised, phase 2 trial. *Lancet Oncol* (2020) 21(1):162–74. doi: 10.1016/S1470-2045(19)30684-9

18. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell* (2015) 161(5):1215–28. doi: 10.1016/j.cell.2015.05.001

19. Nicolosi P, Ledet E, Yang S, Michalski S, Freschi B, O'Leary E, et al. Prevalence of germline variants in prostate cancer and implications for current genetic testing guidelines. *JAMA Oncol* (2019) 5(4):523–8. doi: 10.1001/jamaoncol.2018.6760

20. Guedes LB, Antonarakis ES, Schweizer MT, Mirkheshti N, Almutairi F, Park JC, et al. MSH2 loss in primary prostate cancer. *Clin Cancer Res* (2017) 23(22):6863–74. doi: 10.1158/1078-0432.CCR-17-0955

21. Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. *Cell* (2015) 163(4):1011–25. doi: 10.1016/j.cell.2015.10.025

22. National Comprehensive Cancer Network. *Prostate cancer early detection*. NCCN. Available at: https://www.nccn.org/professionals/physician_gls/pdf/prostate_detection.pdf (Accessed January 14, 20, 2022).

23. Lynch HT, Fusaro RM, Lynch PM. Sebaceous skin lesions as clues to hereditary non-polyposis colorectal cancer. *J Invest Dermatol* (2006) 126(10):2158–9. doi: 10.1038/sj.jid.5700534

24. Ponti G, Losi L, Pedroni M, Lucci-Cordisco E, Di Gregorio C, Pellacani G, et al. Value of MLH1 and MSH2 mutations in the appearance of Muir-torre syndrome phenotype in HNPCC patients presenting sebaceous gland tumors or keratoacanthomas. *J Invest Dermatol* (2006) 126(10):2302–7. doi: 10.1038/sj.jid.5700475

25. Mangold E, Pagenstecher C, Leister M, Mathiak M, Rütten A, Friedl W, et al. A genotype-phenotype correlation in HNPCC: strong predominance of msh2 mutations in 41 patients with Muir-torre syndrome. *J Med Genet* (2004) 41(7):567–72. doi: 10.1136/jmg.2003.012997

26. Graham LS, Montgomery B, Cheng HH, Yu EY, Nelson PS, Pritchard C, et al. Mismatch repair deficiency in metastatic prostate cancer: Response to PD-1 blockade and standard therapies. *PloS One* (2020) 15(5):e0233260. doi: 10.1371/journal.pone.0233260

27. Henderson A, Langley SE, Laing RW. Is bicalutamide equivalent to goserelin for prostate volume reduction before radiation therapy? A prospective, observational study. *Clin Oncol (R Coll Radiol)* (2003) 15(6):318–21. doi: 10.1016/s0936-6555(03)00093-1

28. Majumder K, Brandberg Y, Johansson H, Castellanos E, Ullen A, Lennernas B, et al. Effect on prostate volume following neoadjuvant treatment with an androgen receptor inhibitor monotherapy versus castration plus an androgen receptor inhibitor in prostate cancer patients intended for curative radiation therapy: A randomised study. *Mol Clin Oncol* (2018) 8(1):141–6. doi: 10.3892/mco.2017.1487

29. Mason M, Maldonado Pijoan X, Steidle C, Guerif S, Wiegel T, van der Meulen E, et al. Neoadjuvant androgen deprivation therapy for prostate volume reduction, lower urinary tract symptom relief and quality of life improvement in men with intermediate- to high-risk prostate cancer: A randomised non-inferiority trial of degarelix versus goserelin plus bicalutamide. *Clin Oncol (R Coll Radiol)* (2013) 25(3):190–6. doi: 10.1016/j.clon.2012.09.010

30. Mercader M, Bodner BK, Moser MT, Kwon PS, Park ES, Manecke RG, et al. T Cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. *Proc Natl Acad Sci U S A* (2001) 98(25):14565–70. doi: 10.1073/pnas.251140998

31. Gannon PO, Poisson AO, Delvoye N, Lapointe R, Mes-Masson AM, Saad F. Characterization of the intra-prostatic immune cell infiltration in androgen-deprived prostate cancer patients. *J Immunol Methods* (2009) 348(1-2):9–17. doi: 10.1016/j.jim.2009.06.004

32. Sorrentino C, Musiani P, Pompa P, Cipollone G, Di Carlo E. Androgen deprivation boosts prostatic infiltration of cytotoxic and regulatory T lymphocytes and has no effect on disease-free survival in prostate cancer patients. *Clin Cancer Res* (2011) 17(6):1571–81. doi: 10.1158/1078-0432.CCR-10-2804

33. Mourey L, Piulats JM, Appleman LJ, Romano E, Gravis G, Gurney H, et al. Pembrolizumab plus docetaxel and prednisone in patients with metastatic castration-resistant prostate cancer: Long-term results from the phase 1b/2 KEYNOTE-365 cohort b study. *Eur Urol* (2022) 82(1):22–30. doi: 10.1016/j.eururo.2022.02.023

34. Sommer U, Ebersbach C, Beier AK, Baretton GB, Thomas C, Borkowetz A, et al. Influence of androgen deprivation therapy on the PD-L1 expression and immune activity in prostate cancer tissue. *Front Mol Biosci* (2022) 9:878353. doi: 10.3389/fmolb.2022.878353

35. Merck. (2023). Available at: https://www.merck.com/news/merck-announces-keynote-991-trial-evaluating-keytruda-pembrolizumab-plus-enzalutamide-and-androgen-deprivation-therapy-in-patients-with-metastatic-hormone-sensitive-prostate-cancer-to-stop-for/ (Accessed February 5, 2023).

36. Cercek A, Lumish M, Sinopoli J, Weiss J, Shia J, Lamendola-Essel M, et al. PD-1 blockade in mismatch repair-deficient, locally advanced rectal cancer. *N Engl J Med* (2022) 386(25):2363–76. doi: 10.1056/NEJMoa2201445

37. Chalabi M, Verschoor YL, van den Berg J, Sikorska K, Beets G, Lent AV, et al. Neoadjuvant immune checkpoint inhibition in locally advanced MMR-deficient colon cancer: The NICHE-2 study. *Ann Oncol* (2022) 33(Suppl 7):S808–69.

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 22 March 2023
DOI 10.3389/fonc.2023.1147590

Check for updates

OPEN ACCESS

EDITED BY
Inge Bernstein,
Aalborg University Hospital, Denmark

REVIEWED BY
Martyna Filipska,
Fundació Institut Mar d'Investigacions Mèdiques (IMIM), Spain
Fiona Lalloo,
Manchester University NHS Foundation Trust (MFT), United Kingdom

*CORRESPONDENCE
Shizuko Sei
seis@mail.nih.gov
Steven M. Lipkin
stl2012@med.cornell.edu
Matthias Kloor
Matthias.Kloor@med.uni-heidelberg.de

SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 18 January 2023
ACCEPTED 09 March 2023
PUBLISHED 22 March 2023

CITATION
Sei S, Ahadova A, Keskin DB, Bohaumilitzky L, Gebert J, von Knebel Doeberitz M, Lipkin SM and Kloor M (2023) Lynch syndrome cancer vaccines: A roadmap for the development of precision immunoprevention strategies. *Front. Oncol.* 13:1147590. doi: 10.3389/fonc.2023.1147590

COPYRIGHT
© 2023 Sei, Ahadova, Keskin, Bohaumilitzky, Gebert, von Knebel Doeberitz, Lipkin and Kloor. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Lynch syndrome cancer vaccines: A roadmap for the development of precision immunoprevention strategies

Shizuko Sei[1]*, Aysel Ahadova[2,3], Derin B. Keskin[4,5,6,7,8,9], Lena Bohaumilitzky[2,3], Johannes Gebert[2,3], Magnus von Knebel Doeberitz[2,3], Steven M. Lipkin[10]* and Matthias Kloor[2,3]*

[1]Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Rockville, MD, United States, [2]Department of Applied Tumor Biology, Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany, [3]Clinical Cooperation Unit Applied Tumor Biology, German Cancer Research Center Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany, [4]Translational Immunogenomics Laboratory, Dana-Farber Cancer Institute, Boston, MA, United States, [5]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, United States, [6]Broad Institute of The Massachusetts Institute of Technology (MIT) and Harvard, Cambridge, MA, United States, [7]Department of Computer Science, Metropolitan College, Boston University, Boston, MA, United States, [8]Harvard Medical School, Boston, MA, United States, [9]Section for Bioinformatics, Department of Health Technology, Technical University of Denmark, Lyngby, Denmark, [10]Joan and Sanford I. Weill Department of Medicine, Weill Cornell Medical College, New York, NY, United States

Hereditary cancer syndromes (HCS) account for 5~10% of all cancer diagnosis. Lynch syndrome (LS) is one of the most common HCS, caused by germline mutations in the DNA mismatch repair (MMR) genes. Even with prospective cancer surveillance, LS is associated with up to 50% lifetime risk of colorectal, endometrial, and other cancers. While significant progress has been made in the timely identification of germline pathogenic variant carriers and monitoring and early detection of precancerous lesions, cancer-risk reduction strategies are still centered around endoscopic or surgical removal of neoplastic lesions and susceptible organs. Safe and effective cancer prevention strategies are critically needed to improve the life quality and longevity of LS and other HCS carriers. The era of precision oncology driven by recent technological advances in tumor molecular profiling and a better understanding of genetic risk factors has transformed cancer prevention approaches for at-risk individuals, including LS carriers. MMR deficiency leads to the accumulation of insertion and deletion mutations in microsatellites (MS), which are particularly prone to DNA polymerase slippage during DNA replication. Mutations in coding MS give rise to frameshift peptides (FSP) that are recognized by the immune system as neoantigens. Due to clonal evolution, LS tumors share a set of recurrent and predictable FSP neoantigens in the same and in different LS patients. Cancer vaccines composed of commonly recurring FSP neoantigens selected through prediction algorithms have been clinically evaluated in LS carriers and proven safe and immunogenic. Preclinically analogous FSP vaccines have been shown to elicit FSP-directed immune responses and exert tumor-preventive efficacy in murine models of LS. While the immunopreventive efficacy of "off-the-shelf" vaccines consisting of commonly recurring FSP antigens is currently investigated in LS clinical trials, the feasibility and utility of personalized FSP vaccines with

individual HLA-restricted epitopes are being explored for more precise targeting. Here, we discuss recent advances in precision cancer immunoprevention approaches, emerging enabling technologies, research gaps, and implementation barriers toward clinical translation of risk-tailored prevention strategies for LS carriers. We will also discuss the feasibility and practicality of next-generation cancer vaccines that are based on personalized immunogenic epitopes for precision cancer immunoprevention.

KEYWORDS

lynch syndrome, DNA mismatch repair deficiency, microsatellite instability, frameshift mutations, tumor neoantigens, cancer vaccines, immunoprevention, precision cancer prevention

Introduction

Cancer prevention strategies are generally centered around the reduction of cancer risks. Modifiable cancer risk factors include tobacco use, alcohol consumption, obesity, diabetes, and infection with oncogenic viruses, such as human papillomaviruses (HPV) and hepatitis B virus (HBV). Lifestyle changes and receiving prophylactic vaccines against HPV and HBV can significantly reduce these risks (1). In contrast, genetic predisposition to cancer is not modifiable. Individuals with hereditary cancer syndromes (HCS) account for 5 to 10% of all cancer cases (2). They are clinically identifiable by genetic testing (3–5), are well characterized with predictable ages of disease onset, organ involvement, and molecular pathophysiology, and can be closely monitored for early cancer detection and diagnosis based on HCS guidelines (6). Cancer risk mitigation strategies for HCS carriers, therefore, include primary prevention of cancer as well as detection and elimination of cancer precursors and early-stage (*in situ*) cancers before they progress to invasive cancers, the approach referred to as cancer interception (7). While much progress has been made in the development of new or improved methods of detecting cancer early (8), with the exception of aspirin for Lynch syndrome (LS) (9) there are currently no effective cancer preventive or interceptive approaches available to them other than surgical (endoscopic or surgical) removals. Most of the conventional anticancer therapeutic agents are too toxic for cancer interception.

Abbreviations: HCS, Hereditary cancer syndromes; LS, Lynch syndrome; MMR, DNA mismatch repair; FSP, Frameshift peptides; TME, Tumor microenvironment; ICI, Immune checkpoint inhibitors; cMS, Coding microsatellites; MMRd, DNA mismatch repair deficiency; CRC, Colorectal cancer; rFSP, Recurring FSP; CMMRD, Constitutional mismatch repair deficiency; Indel, Insertion-deletion; HNPCC, Hereditary non-polyposis colorectal cancer; IHC, Immunohistochemistry; MSI, Microsatellite instability; cfDNA, Cell-free tumor DNA; TILs, Tumor infiltrating lymphocytes; NSAIDs, Non-steroidal anti-inflammatory drugs; saRNA, self-amplifying RNA; circRNA, circular RNA; B2M, Beta-2-Microglobulin; MDSC, Myeloid derived suppressive cells; T_{RM}, Tissue-resident memory CD8+ T cells; ICR, Immune checkpoint receptors.

Vaccine-preventable infectious diseases (10) are a good example of illnesses that can be safely prevented if vaccines are used as recommended. The unprecedented speed of successful development and deployment of COVID-19 mRNA vaccines in 2020 ~ 2021 was a tremendous scientific achievement that had culminated from years of research in relevant scientific disciplines, including coronavirus virology, vaccinology, and innovative mRNA vaccine technology, and a strong public-private partnership (11, 12). Prerequisites for successful vaccine development generally include identification and characterization of causative agents, understanding of disease pathogenesis and pathophysiology, and availability of suitable preclinical tools and animal models, which recapitulate human disease conditions and host immune responses and therefore can provide a proof of principle for *in vivo* vaccine efficacy. Compared to prophylactic vaccines against infectious pathogens that have generally fulfilled these prerequisites throughout the history of vaccine development (13), the development of cancer vaccines in general has been met with more challenges (14).

The effect of cancer vaccine was first evaluated in cancer patients in 1959 (15) shortly after Burnet postulated the concept of cancer immunosurveillance (16). While important advancements were made in cancer immunology and vaccinology over the last several decades, the majority of cancer vaccine research has focused on eliciting effective antitumor immunity to treat advanced cancer (17). Various therapeutic cancer vaccines were extensively evaluated in patients with advanced cancer with little success, likely due to the immunosuppressive factors locally in the tumor microenvironment (TME) and systemically (18). The concept of cancer vaccines for immunoprevention started gaining more traction in the last 20 years owing to the pioneering work of Finn, Disis, and others against non-viral cancers (14, 19–25). Target antigens selected for cancer preventive vaccines have predominantly been tumor-associated or tumor-specific antigens that are overexpressed or specifically expressed in cancer precursors and cancer cells and proven immunogenic across different HLA types (26, 27). Cancer vaccines with such commonly expressed (shared) tumor antigens can be more easily studied for efficacy in a well-defined high-risk cohort and thus can be streamlined for further development.

More recently, successful immunotherapy outcomes with immune checkpoint inhibitors (ICI) for various cancers have clearly shown that the immune system can mount strong antitumor immune responses leading to complete remission in some cases if systemic and local immunosuppression in the TME is effectively blocked (28, 29). Interestingly, antitumor immune responses unleashed by immune checkpoint blockade have been shown to target a large repertoire of tumor antigens that are unique to individual patients (i.e., personalized antigens) (30–32). It is conceivable that more robust and durable antitumor immunity can be elicited by cancer vaccines in the prevention or interception setting, wherein local immunity in the TME is less compromised and there is still low clonal heterogeneity of tumor antigens (22, 33, 34). Naturally, questions arise as to whether immunopreventive cancer vaccines can be developed based on personalized tumor antigens and whether such personalized vaccines are more efficacious and desirable than cancer vaccines that target shared/common tumor antigens. This review will discuss the development of LS vaccines as a model strategy for preventing cancer in HCS cohorts, emerging enabling technologies, research gaps and implementation barriers for cancer immunoprevention, and research trajectory towards next-generation precision cancer vaccines for immunoprevention.

Precision cancer prevention

Apart from the modifiable risk reduction strategies discussed earlier, cancer prevention for high-risk cohorts can be improved by determination of risks based on oncogenic mechanisms inherent to a specific cohort, closer monitoring of affected individuals for early cancer detection, and timely and effective interventions developed specifically for each high-risk group. These risk-tailored cancer prevention strategies are interchangeably referred to as personalized or precision cancer prevention. For the purpose of this review, which is focused on cancer prevention strategies for HCS cohorts, in particular LS, we define precision cancer prevention as risk-tailored cancer prevention strategies informed by underlying oncogenic mechanisms responsible for the development and progression of cancer and molecular alterations targetable for cancer prevention and interception in high-risk populations (35). We will use the term "personalized" when we refer to tumor antigens unique to each individual as opposed to shared or commonly expressed antigens (36).

The concept of precision oncology was originally introduced as cancer genomics-informed "personalized or precision" cancer medicine to facilitate the decision on treatment choices for individual cancer patients (37). The common denominators of precision cancer prevention and precision cancer medicine strategies are the involvement of molecular and immune mechanisms of oncogenesis in the decision-making process for interventions rationally and uniquely developed for individuals. Neoantigens are newly acquired and expressed "non-self" antigens arising from gene mutations, exogenous genes (e.g., viral proteins), or alternative antigen processing. The host immune system recognizes these neo-peptides presented with MHC molecules on the cell surface as non-self, mount immune responses against them, and eliminate the neoantigen-expressing aberrant cells from the body (38). During tumor development and progression, tumors accumulate numerous gene mutations, which, if translated, give rise to neoantigens (39, 40). These neoantigens expressed in cancers can be targeted by the host immune system for surveillance and elimination. Although it's been long postulated that tumor neoantigens would serve as promising cancer vaccine antigens, the discovery and neoepitope selection was a major hurdle until recently. The advent of next-generation sequencing technologies and rapid development of powerful computational analytical tools, which enable comprehensive "mutanome" analysis of individual tumors and the identification of personalized immunogenic neoepitopes, has led to seminal neoantigen cancer vaccine studies in melanoma patients either as peptide-based (41) or mRNA-based vaccines (42). Both studies demonstrated immunized patients mounted robust T cell responses to unique neoantigens, associated with prolonged and objective responses in some cases. The long-term outcome study of patients who received personal neoantigen vaccines demonstrated the clinical benefit at a median follow-up of 4 years post-vaccination and long-term persistence of memory T cells specific to personal neoantigens as well as the evidence of epitope spreading (43).

While there is mounting evidence to suggest that immune responses directed against personal neoantigens can block or control cancer growth and clinically benefit vaccinated patients (30–32, 41–43), the approach cannot be generally translated into the prevention setting unless tumor neoantigens could be "predicted" in individuals who have yet to develop cancers. The first breakthrough observation was made by Kloor, von Knebel Doeberitz, and colleagues, who demonstrated that insertion/deletion frameshift (FS) mutations could be predicted based on the known genetic sequences of coding microsatellites (cMS) and that the specific mutation frequencies could be evaluated in LS/mismatch repair deficiency (MMRd)-associated tumors (44, 45). They discovered that colonic adenomas from LS carriers harbored MMRd-driven FS mutations in the cMS regions at high frequencies for certain genes at levels similar to those found in colorectal carcinomas (46). They further demonstrated frameshift peptides (FSP)-specific T cell responses could be observed not only in LS patients with colorectal cancer (CRC), but also in cancer-free asymptomatic LS carriers (47). These findings clearly demonstrated that LS carriers harbored FSP neoantigens before the onset of overt CRC tumorigenesis and the host immune system was capable of mounting anti-FSP immunity, which may play a role in keeping tumor growth in check. Using commonly recurring (broadly shared) FSP (rFSP) neoantigens as vaccine antigens, they subsequently proposed an rFSP neoantigen-based cancer vaccine for MMRd cancers and successfully demonstrated the safety and immunogenicity in Phase I/IIa clinical trial (48), providing the proof of principle of rFSP neoantigen-based cancer vaccine strategies for cancer prevention and interception in LS carriers.

Tumor-specific neoantigens can be vastly heterogenous and immune responses are restricted by MHC molecules (38). Their expression levels also vary among different neoantigens. Because of the intra- and inter-individual heterogeneity of tumor-specific

neoantigens and the diversity of immune responses that are determined by HLA alleles, it is extremely challenging, if not impossible, to develop broadly applicable cancer vaccines targeting shared neoantigens for different HCS carriers, with LS being an exception as discussed earlier. In this regard, the development of personalized cancer preventive vaccines may be more straightforward. Similar to the approach used for the development of precision cancer therapeutic vaccines (41, 42), personalized immunogenic epitopes for preventive vaccines can be identified from molecular and immuno-neoepitope analysis of precancerous lesions. The question, however, is whether there is an advantage to personalize FSP-based cancer preventive vaccines for individual LS carriers when shared FSP antigens can be readily identified. Considering the amount of time and resources required to generate such personalized FSP vaccines for nearly one million LS carriers in the US alone, the concept of personalized immunopreventive cancer vaccines is prohibitively impractical for the LS cohort at this time. At the same time, the debate on the use of personalized neoantigen based vaccines for cancer prevention should also involve the lifetime disease severity and progression trajectory. For example, consider children with constitutional mismatch repair deficiency (CMMRD) syndrome, one of the most aggressive forms of childhood cancer predisposition syndromes, resulting from biallelic deleterious germline mutations in the MMR genes (49). As in LS, DNA MMR deficiency can trigger FS mutations in the cMS, giving rise to FSP neoantigens in these children (50). Children with CMMRD develop brain tumors, hematological malignancies (in particular, non-Hodgkin lymphomas of T-cell lineage, T cell ALL and AML), gastrointestinal and other LS-associated cancers, sarcomas (e.g., osteosarcoma and rhabdomyosarcoma), and other childhood cancers (e.g., neuroblastoma and Wilms tumor) (49, 51). Cancers arising in children with CMMRD have the highest mutational and MS insertion-deletion (MS-indel) burden, are resistant to chemo-radiation interventions, and considered lethal (52). Children with CMMRD therefore may clinically benefit from receiving personalized cancer preventive vaccines. If we aim to develop and deploy risk-tailored and risk-weighted precision cancer prevention strategies, the critical first step is to identify the genetic predisposition carriers and investigate the pathophysiology of oncogenesis in each HCS population.

Advances in genetic predisposition screening technologies

Of the ~140,000 new diagnoses of CRC each year in the United States, ~25% to 30% of patients diagnosed have a first or second degree relative (parents, siblings, children, uncles, aunts and first cousins) with CRC (53). The most common inherited CRC syndromes is LS, which is diagnosed by germline autosomal dominant mutations of DNA MMR genes, *MLH1, MSH2, MSH6 and PMS2*, or structural variations in *EPCAM* that drive MSH2 epigenetic inactivation (54, 55). LS is estimated to occur in approximately 1:280 individuals (56). A related syndrome is CMMRD discussed above, a much less frequent pediatric autosomal recessive disease where children inherit bi-allelic MLH1, MSH2, MSH6 or PMS2 mutations that drive aggressive cancer predisposition and are affected with cancers as often as every 2-3 years in early life and commonly perish from brain, GI, and hematopoietic malignancies (50). Additionally, autosomal recessive mutations of MMR genes, MSH3 or MLH3, cause colorectal polyposis and CRC, which is a separate syndrome characterized by distinct patient phenotype and familial inheritance pattern (57–60). This review section focuses on LS.

Historically, LS was referred to as hereditary non-polyposis colorectal cancer (HNPCC). However, LS is now preferred to highlight that that these patients and their families have higher rates of multiple other cancers, most notably endometrial and gastric cancers, but also including ovarian, pancreatic-biliary, urinary tract (kidney, renal pelvis, ureter, bladder, and prostate), small intestinal, brain cancers and sebaceous neoplasms of the skin, among others (61).

Overall, there are two primary strategies for diagnosis of LS. Historically, family history followed by germline DNA mutation testing performed on patients with personal or family history of cancer suspicious for LS was the primary approach. This approach uses different clinical criteria, most notably the Amsterdam or Bethesda criteria (53), both of which focus on age on onset for CRC, and family cancer history of first-, second- and third-degree relatives for LS associated cancers. However, family history taking, while virtually costless and in principle universally implemented, was found to significantly underdiagnose LS (62–65). In part, at least in the United States, this is driven by growing provider economic and corporate medical pressures on primary care physicians to rapidly provide comprehensive medical care for ever growing panels of patients (which can average 1 patient every 15 minutes in some clinics anecdotally) and primarily focus on symptomatic crises rather than less urgent preventative medical care that shortchange history and disease interception strategies. Additionally, there are non-trivial rates of non-paternity, which range from ~0.4% to as high as 30% in different populations but roughly averaging to ~1% across different societies (66, 67), further confounding family history taking. Most importantly, population screening studies of CRC and endometrial cancer (68, 69), primarily by immunohistochemistry (IHC) testing, provided direct data revealing that many LS patients had limited family history and/or later onset cancers. In addition to the practical health services implementation issues with family history taking discussed above, this is driven by the fact that family history does not capture patients who have *de novo* LS mutations (aka, a new mutation not inherited from either biological parent) and because many LS patients carry MSH6 or PMS2 mutations, which confer lower overall lifetime cancer risk than MLH1 or MSH2 mutation carriers (54, 56).

Underdiagnosis of LS is not unique to the United States. It also remains a challenging issue in Europe (70, 71). A Swedish study has demonstrated that one third of LS patients referred for genetic testing already had cancer, indicating that these individuals' genetic risk was unknown until they developed LS-associated cancers (72). This proportion of individuals diagnosed with LS due to cancer diagnosis has not changed over the decades (72). A survey across 14

Western European countries showed that the quality of family history taking was thought to be generally poor and there were virtually no specific campaigns or strategies in place to increase the public awareness of hereditary cancers except in one country (Germany) (73). Another study has also reported that family history has been poorly documented even in the electronic health record (74) contributing to the deficiency in family history taking approach. Thus, while personal and family history taking approach has been the cornerstone of LS diagnosis, it significantly undercounts its prevalence.

The second primary approach to LS diagnosis starts not with clinical criteria per se, but molecular screening of CRC, endometrial, pancreatic and other tumor specimens for evidence of MMRd from patients who are diagnosed with carcinomas. This can be performed as polymerase chain reaction (PCR)-based microsatellite instability (MSI) testing of newly or previously archived tumors after diagnosis, immunohistochemistry (IHC) for MMR genes, or direct tumor gene panel sequencing and mutation burden analysis to identify patients who then have germline testing for MMR gene mutations (which is sometimes but not always done simultaneously with tumor sequencing) (54). Importantly, these molecularly initiated approaches also identify patients who have sporadic (aka non-LS) MMRd tumors, which arise *via* somatic (non-CMMRD congenital) bi-allelic mutation and/or epigenetic inactivation of MMR genes, primarily MLH1 (54).

Cascade testing involves targeted mutation analysis testing of blood relatives from patients who are affected by a genetic disease (4, 75–77). Recent clinical trial and cost effectiveness research studies have provided evidence that primary molecular screening augmented by follow-up targeted cascade testing of family members of affected LS (and other genetic disease) probands is a cost effective public health strategy to diagnose a high percentage of LS mutation carriers that is predicted to identify almost all affected individuals after approximately a decade of implementation (4, 75–77).

Analysis of circulating cell-free tumor DNA (cfDNA) using liquid biopsy has enabled non-invasive detection of tumor mutations. Currently, liquid biopsy is an established technology to replace and/or augment tumor biopsy sequencing, including the detection of minimal residual disease (MRD) after surgery or chemotherapy. Liquid biopsy can be used for detection of LS germline mutations (78) and for detecting MMRd tumors, which carry very elevated tumor mutation burdens. Currently, the highest sensitivity for liquid biopsy detection of MMRd tumor DNA is shown by low pass whole genome sequencing of cfDNA and mutation signature analysis (79), which draws on sequence data from low pass coverage sampling of the ~23,000,000 microsatellites encoded in the human genome (50). However further studies, and perhaps additional augmentation by orthogonal technologies including protein and microbial analyte data streams, are needed before this population surveillance strategy becomes useful for LS diagnosis, screening, and surveillance.

Because of the high tumor mutation burden, it has been long been appreciated that MMRd tumors have elevated numbers of tumor infiltrating lymphocytes (TILs) and other enriched histopathology features of the tumor microenvironment (54). Recently, there has been evidence that machine learning of histopathology images of tumors can detect "MMRdness (80)". However, although innovative and expected to improve in the future with advances in computational analysis, the sensitivity and specificity of ML histopathology detection of MMRd status may currently be problematic and not just yet ready for clinical application (81).

In summary, identification of LS is important because it is a clinically actionable diagnosis driving increased tumor surveillance, chemoprevention [primarily aspirin, discussed at length elsewhere (9)] and post-tumor therapy choice [immune checkpoint inhibition, discussed at length elsewhere (82)] in a high-risk cancer population. Screening and diagnosis of LS mutation carriers is an evolving paradigm that begins with personal and family history as its cornerstone, but requires additional molecular, computational and health services analyses to reach its potential for improving survival for affected patients. This point is particularly true regarding underrepresented and underserved minority populations, which often have lower rates of information collection or access from detailed family history taking, in addition to less access to preventative medical care and cost-intensive medical technologies (62–65).

Lynch syndrome vaccines for immunoprevention

LS is an ideal model disease for testing the potential of cancer immunopreventive approaches due to the pathogenesis of LS-associated tumors. The penetrance of the disease varies widely depending on the MMR gene affected in the germline, with *MLH1* and *MSH2* genes being associated with relatively high cancer risk (about 50%: Prospective Lynch Syndrome Database or PLSD) (83) and found in about 90% of LS-associated tumors. In addition to the affected MMR gene, further factors are suspected to influence individual cancer risk in LS carriers, as also within-gene and even within-family differences in cancer risk have been observed. These factors include other genetic constellations (polygenic risk score) and environmental influences. More recently, the possible influence of the immunological factors has also been gaining substantial attention, which is related to the growing knowledge about a close interplay between arising MMR-deficient tumor cell clones and the immune microenvironment.

The driving force of carcinogenesis in LS is MMRd, leading to the inability of affected cells to repair base mismatches occurring during DNA replication. When unrepaired, such errors cause indel mutations, typically indels of one or two nucleotides at short repetitive DNA stretches (microsatellites). The molecular phenotype of MMR-deficient cells is characterized by the accumulation of insertion/deletion mutations at microsatellites and called MSI as discussed earlier. Microsatellite regions are distributed over the entire human genome. Because approximately 99% of the genome has no protein-coding function, most microsatellite mutations do not have an immediate effect on a cell's functional phenotype. However, mutations at microsatellites in protein-coding genomic regions

(coding microsatellites, cMS), which are mostly mononucleotide repeats, can have drastic consequences on the function of the encoded protein and its immunological properties. Due to an insertion or a deletion of one or two nucleotides, the entire subsequent reading frame is shifted (frameshift mutations). This can lead to premature stop codons and translation of truncated, non-functional proteins. cMS mutations affecting tumor suppressor genes can drive tumorigenesis in the mutated cell. Simultaneously, the newly translated FSP sequence often contains numerous epitopes, which are foreign to the host's immune system, rendering the affected cells highly immunogenic.

Thus, the carcinogenic process of LS cancers is tightly linked to the generation of highly immunogenic antigens. This provides a basis for immune-modulatory therapies, e.g., using immune checkpoint inhibitors, which can reactivate a pre-existing, but exhausted immune response. However, for a largely applicable preventive approach, the predictability of the antigens plays a crucial role. The Darwinian selection principles behind the evolution of MSI cancers allow the prediction of antigens before a cancer develops (Figure 1A). As microsatellite mutations that confer proliferation and growth advantage (by disabling tumor suppressor genes while evading immune recognition and elimination) will be selected for, the respective antigens are over-represented in cancer precursors and manifest cancers and thus serve as promising vaccine targets.

Using a comprehensive bioinformatics approach, we and others previously characterized cMS across the human genome, establishing two major findings: (1) the mutation frequency of a microsatellite largely depends on its length, following a sigmoid curve; (2) cMS mutations with a frequency higher than what was predicted based on microsatellite length are the likely drivers of tumorigenesis, reflecting selection during MSI cancer evolution. Using the information about length-adjusted mutation frequency, it is possible to predict the relevance of specific mutations in the carcinogenic process and their frequencies.

Using this approach, we were able to trace the evolution of MSI cancers down to a few recurrent mutations shared across tumors and patients, opening a new avenue in the field of cancer prevention. By identifying key driver mutations in the MSI carcinogenic process, we predicted the resulting peptide structures resulting from these mutations and demonstrated their ability to induce T cell responses *in vitro* (47, 84) (Figure 1B). In the next step, these candidates were combined in a trivalent vaccine containing three recurrent and immunogenic antigens derived from AIM2 (-1 deletion), TAF1B (-1 deletion) and HT001 (-1 deletion) frameshift cMS mutations that were shared by more than 85% of MSI tumors. This vaccine was evaluated in a first-in-human Phase I/IIa clinical trial analyzing the safety and immunogenicity of a cancer vaccine in a total of 22 patients (48). The study demonstrated a favorable safety profile with no treatment-related severe systemic adverse effects observed in any of the vaccinated patients. However, grade 2 local injection site reactions have been observed in 3/22 vaccinated study participants, indicating that vaccination-related side effects need to be accounted for in future vaccine formulations and strategies, particularly in LS carriers with pre-existing FSP-specific immune responses. Importantly, all patients vaccinated per protocol demonstrated FSP-specific cellular (predominantly CD4 T cells) and humoral immune response against at least one vaccine antigen.

Although the results of the first clinical trial with the trivalent FSP peptide vaccine described above were highly encouraging,

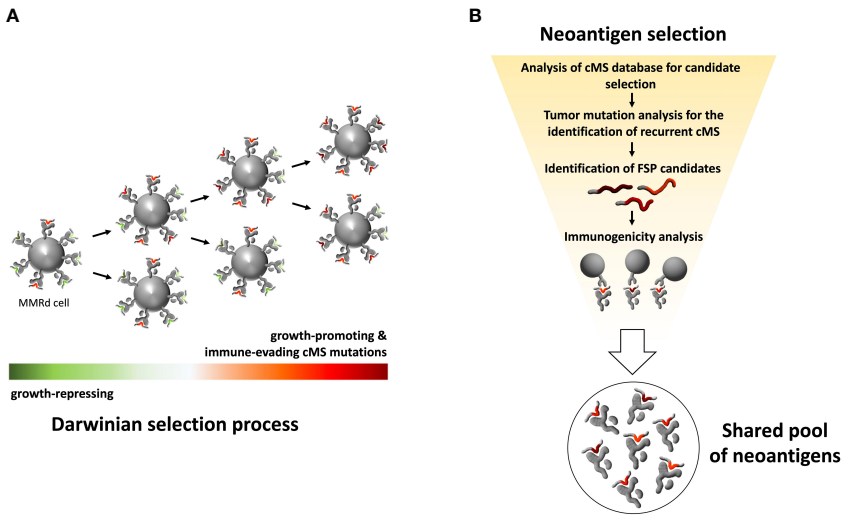


FIGURE 1
Schematic illustration of MMRd tumor clone selection process and workflow for selecting a pool of shared immunogenic neoantigens. **(A)** Schematic illustration of Darwinian selection process underlying MMRd cancer evolution. The random accumulation of indel mutations in cMS, caused by impairment of the MMR system, is followed by a non-random persistence of mutations. Cell clones carrying mutations that promote tumor outgrowth or provide other survival advantages, such as immune evasion, are positively selected. This evolutionary selection of mutations leads to recurrent cMS mutation patterns in MMRd cancers and thus a predictable pool of FSPs. **(B)** Strategy for the selection of shared, immunogenic FSPs for immunoprevention. Screening of a genome-wide cMS database forms the basis for the identification of recurrent cMS mutations shared by MMRd cancers. Accounting for mutation frequency in MMRd cancers, immunogenicity prediction in silico and immunogenicity testing *in vitro*, a pool of candidate neoantigens can be selected for FSP vaccines.

antitumor efficacy was not the primary objective, which would have required a long-term follow-up if tumor recurrence was used as the primary endpoint. To ask whether the FSP-based cancer vaccine could prevent or intercept CRC tumorigenesis, pre-clinical studies in mouse models have been performed. Based on the *VCMsh2* LS mouse model developed by Kucherlapati and Edelman et al. (85), which recapitulates human LS-associated intestinal tumorigenesis by biallelic Villin-dependent conditional knockout of *Msh2* in the entire intestinal epithelium, the preventive effect of rFSP vaccination was evaluated. Bioinformatics analysis of 488,235 cMS in the murine genome combined with the gene expression and mutation frequency data identified thirteen candidates possibly relevant for the MSI tumorigenesis in Lynch mice (86). The immunological assessment including epitope prediction and immunogenicity analysis revealed four promising candidates for vaccination. Vaccination with these candidates, Nacad (-1 deletion), Maz (-1 deletion), Senp6 (-1 deletion), and Xirp1 (-1 deletion) alone or in combination with non-steroidal anti-inflammatory drugs (NSAIDs) (aspirin or naproxen), which have been examined for chemo preventive effects in LS patients (9, 87, 88), elicited robust T cell immune responses as measured by IFNγ ELISpot and a significant tumor-preventive effect in *VCMsh2* mice. Interestingly, the tumor-preventive effect was strongest in the rFSP vaccine plus naproxen combination arm, supporting the hypothesis that NSAIDs may enhance vaccine-induced antitumor efficacy by reshaping the immune microenvironment in the intestinal mucosa and enhancing immune surveillance (89, 90). Clinically, this suggests that the reduction of tumor incidence by NSAIDs, reported for Lynch carriers in retrospective (91) and controlled prospective studies (9, 87, 88), could further be enhanced by FSP vaccines.

In addition to the peptide based FSP vaccination approach, further studies pursuing a different vaccination approach are currently underway, including viral vector-based FSP antigen delivery with a substantially higher number of antigens (over 200), which have been derived from human MSI tumors as analyzed by *in vitro, in vivo* and *ex vivo* tools. The study is currently recruiting LS patients for evaluation in clinical trials (92).

HLA genotype and tumor antigen evolution

Both clinical and preclinical data on FSP vaccines' effectiveness hinted at the importance of epitope selection for eliciting CD4 or CD8 T cell responses. Although the dominant role of cytotoxic CD8 T cell response has been suggested in the efficacy of tumor cell elimination, immune responses engaging CD4 T cell response have been gaining more relevance with growing knowledge in tumor immunology (93, 94). Specifically, the human trivalent FSP vaccine trial (48) demonstrated that FSP-specific immune responses were predominantly mediated by CD4-positive T cells, whereas less than 50% of vaccinated patients developed significant CD8-positive T cell responses. Although this may in part be related to the use of long peptides and Montanide ISA51 used as an adjuvant, the response pattern may also reflect the availability of epitopes in the FSP sequences compatible with the HLA genotype of vaccinated individuals.

The induction of cellular immune responses requires the presentation of epitopes through HLA (or MHC) molecules. Whereas CD4-positive T cell receptors interact with HLA class II molecules, CD8-positive T cells interact with HLA class I molecules. As the structure of the HLA molecules determines the binding affinity and presentation of certain epitopes (95, 96), the HLA type of an individual influences the epitope repertoire presentable by tumor cells and antigen-presenting cells. As the repertoire of HLA molecules is large and the HLA class I and HLA class II antigen-encoding gene loci are highly diverse, the clinical efficacy of cancer vaccines will vary depending on an individual's HLA genotype. Observations from the recent COVID-19 pandemic regarding the disease course and responses to vaccination with SARS-COV-2 antigens support this association (97–99). Thus, although the shared nature of the driver cMS mutations among different patients and tumors allows the use of a limited set of recurrent candidate neoantigens, response to vaccination and tumor-preventive effectiveness might be substantially improved by the adaptation of the epitopes derived from these neoantigens to the specific HLA type of an individual. Accounting for HLA type specificity is therefore an important task for future immune prevention and interception approaches, potentially enabling higher vaccine effectiveness by individualization of the vaccine formulation to a person's HLA genotype (100).

It is plausible to assume, that HLA genotype influences not only immune responses induced by vaccination, but also natural immune responses in LS carriers prior to and after tumor manifestation (47, 101, 102). If HLA type in fact influences immune surveillance, the HLA genotype may influence the tumor incidence or tumor risk in LS carriers. Studies analyzing the possible effect of the individual HLA type on cancer risk in LS have been initiated (100, 103). The findings expected from these studies will guide the future development of next-generation HLA-adapted individualized neoantigen vaccines.

Significant recent advances in vaccine technology and immunology indicate that individualized vaccine strategies hold potential for future cancer immune prevention and interception. At the same time, this strategy poses a challenge to the production process of vaccines. Even if certain HLA genotypes with similar peptide-binding characteristics can be united under an HLA supertype, vaccine formulations need to be adapted and produced on a relatively short-term. Peptide-based vaccination, though offering a robust and well-studied and evaluated technology, may lack sufficient flexibility for adaptations, particularly in the scenario of therapeutic application in patients with manifest cancer.

The application scenario (preventive, interceptive or therapeutic) also directly affects the maximal possible level of individualization. Preventive applications can maximally target likely antigens derived from predictable mutation events. The selection of candidate epitopes is restricted to the candidate antigens proven highly relevant and immunogenic. Personalization according to the current knowledge would concern the HLA genotype-adjusted epitope selection, although

not the candidate spectrum itself. Such "warehouse" vaccines (104, 105) transferred to the high-risk scenario of LS would focus on the most frequently shared FSP neoantigens adjusted to the predominant HLA alleles (Figure 2). In this setting, peptide-based vaccine may possibly offer a time- and cost-saving solution. In a therapeutic or intercepting approach, the presence of a manifest tumor would enable the analysis of tumor mutanomes and peptidomes (36), binding affinity to the specific HLA molecules of the patient, and immunogenicity, thus enabling the construction of a vaccine based on the given specifics of the tumor and patient. However, this process is time-consuming and may not be ideal for timely initiation of interventions required for cancer interception and treatment.

Throughout the continuum of tumor development, the immune system constantly interacts with emerging tumor cell clones, the process known as tumor immunoediting. When developing immunopreventive or immunotherapeutic approaches, tumor immune evasion should be taken into consideration, as the host immune system will not recognize "escape" tumor clones effectively. The interplay between arising tumor cell clones and host's immune cells and characteristics of tumor evolution have been illustrated by several studies (101, 106–110). Such an interplay is particularly pronounced in the scenario of the highly immunogenic MSI cancers. Among those, the ones with LS background could be exposed to a longer process of immunoediting, as the LS carriers have been reported to present with microscopic lesions with normal histomorphology but lacking MMR protein expression (111). Such lesions were proposed to induce the systemic and local immune responses measurable in the blood and colon of tumor-free LS carriers, respectively (47, 102), suggesting the process initiated long before a clinically detectable tumor. We have previously shown that higher frameshift mutation frequency is correlated with lower immunogenicity of resulting neoantigens, whereas a lower frequency of mutations correlated with highly immunogenic antigens (101). This inverse correlation suggests counter-selection of cell clones expressing highly immunogenic neoantigens according to the tumor immunoediting concept. On the other hand, such counterselection may be negated in cases where antigen presentation machinery is dysregulated. In fact, general alterations of the antigen presentation machinery are common in Lynch-associated cancers and observed at higher frequency in advanced lesions. Such evasion phenomena, including complete breakdown of HLA class I-mediated antigen presentation following mutations of the Beta-2-microglobulin (B2M) gene, can interfere with the effectiveness of vaccines. Immune evasion is considered one of the most important reasons for the limited success of previous cancer vaccine trials with a therapeutic design. Therefore, transferring vaccine approaches towards earlier stages (interception) or entirely to cancer prevention in high-risk individuals marks a paradigm change with high potential for re-shaping the field of anti-cancer vaccines and cancer prevention in general. The complexity of this biological process and limited possibilities for experimental investigation calls for mathematical modeling approaches that could account for possible variables and predict the repertoires of relevant antigens or even model the possible outcomes of immune interception approaches.

Advances in next-generation sequencing together with the development of *in silico* epitope prediction algorithms and *ex vivo*

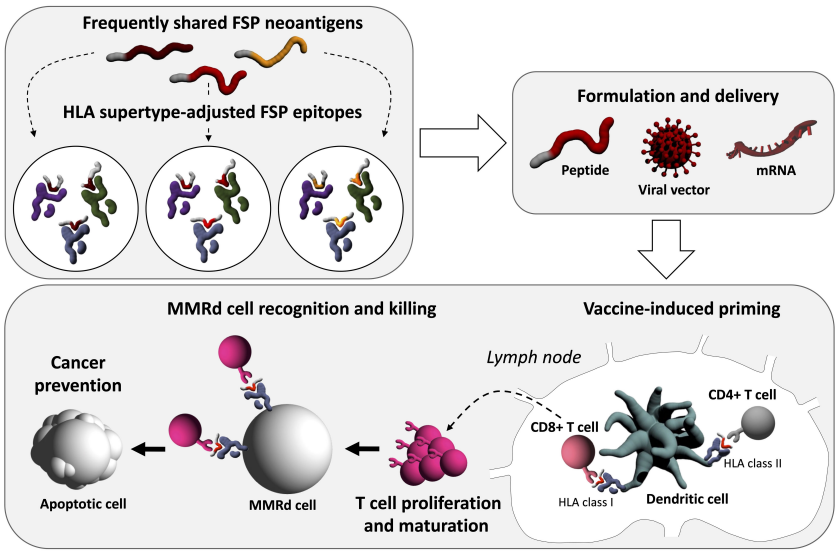


FIGURE 2
Conceptual illustration of next-generation FSP preventive cancer vaccines. After the selection of immunogenic FSP frequently shared among MMRd cells, FSP epitopes can be HLA supertype-adjusted, facilitating higher vaccine effectiveness. Vaccine delivery can be pursued, among others, with different approaches including peptide, viral vector-based, or mRNA technology. Immunologically, the first step will be vaccine-induced priming, during which the delivered neoantigens encounter antigen-presenting cells, most importantly dendritic cells at the injection site. Neoantigen-loaded dendritic cells traffic to lymph nodes which are primary sites of T cell priming. There, vaccine-derived antigens on HLA class I and II molecules are presented to $CD8^+$ and $CD4^+$ T cells. Activated T cells proliferate and mature into effector T cells which leave the lymph nodes entering the periphery. Ideally, neoantigen-specific $CD8^+$ T cells can induce apoptosis in MMRd cells, which present the respective antigen on HLA class I molecules, and thereby prevent cancer.

assessments of immune responses revolutionized the identification of tumor neoepitopes suitable for cancer vaccines (30, 112). Tumor neoantigens originating from genomic alterations beyond those resulting from MMRd-triggered frameshift mutations can also be targeted by the immune system as non-self, leading to tumor-specific T cell responses (32, 113, 114). In fact, vaccination strategies targeting specific tumor neoantigens have demonstrated effective T cell responses against tumor specific antigens and potential clinical benefit (41, 42, 115–117). Neoantigen-based cancer therapies are highly personalized, requiring the development of a vaccine for each individual patient, which limits scalability and availability. To ensure broader applicability, cancer vaccines targeting shared immunogenic neoantigens originating from functionally relevant driver mutations on genes such as KRAS (114, 118), TP53 (119, 120), BRAF (121), PIK3CA (122), and EGFR (123, 124) or from recurrent gene fusions typically occurring in sarcomas (125, 126) have been pursued. However, compared to FSP in LS cancers, the pool of such antigens is limited and their effectiveness in an HLA-diverse population needs to be investigated for other HCS setting. Therefore, the development of personalized cancer vaccines may be warranted for cancer prevention in other HCS carriers, who unlike in LS do not share recurrent neoantigens for "off-the-shelf" vaccine formulations with defined and validated neoantigens. Neoantigen based approaches are promising and likely to improve further with advances in neoantigen prediction pipelines.

Computational tools predictive of peptide binding affinity to specific HLA molecules are available (96, 127–129). These are typically neural network-based algorithms that were trained using existing allele-specific peptides such as those stored in the Immune Epitope Database (130) and Dana-Farber Repository for Machine Learning in Immunology (131). Recently, HLA immune-peptidomics-based approaches to discover HLA-restricted peptides generated large-scale datasets of endogenous HLA-bound peptides that resulted in the development of more accurate epitope prediction algorithms. These algorithms not only predict epitope binding to a specific HLA allele but also consider epitope expression and proteasomal processing to predict epitope presentation more accurately (132). The final frontier in the prediction of neoantigens is the development of an algorithm that can accurately predict antigenicity. Neoantigens can be presented on surface HLA, however not all presented epitopes generate a T cell response, and even presented viral epitopes may not be recognized by T cells (41, 42, 115–117, 133) in some cases. The development of an accurate machine learning algorithm requires thousands of validated T cell epitopes per HLA allele, which are currently unavailable for many alleles. There are ways to overcome this obstacle. Researchers observed more stable HLA ligands yield more immunogenic epitopes (134, 135) and epitopes mimicking pathogen-derived known antigens are more immunogenic (136, 137). Incorporation of these motifs to the neoantigen prediction pipelines may improve vaccine outcomes. Utilization of all these informatics and genomics pipelines may identify validated immunogenic shared neoantigens for LS patients in an HLA-type specific manner that could be the basis of a cancer preventative vaccine. It is plausible that such vaccines may prevent not only LS-associated CRC, but also endometrial and other extracolonic tumors (61, 138) as certain neoantigens are likely shared by the spectrum of cancers arising in LS (Figure 3).

Emerging technologies, research gaps, and translational barriers

Emerging new mRNA-based vaccine technologies (139, 140) have transformed the medical field by offering a technological platform with high adaptive capacity, allowing rapid translation of newly gained genomic knowledge into clinical applications for the prevention and treatment of human diseases (42, 141–143). In addition to its high immunogenicity, flexibility and versatility, relatively straight forward regulatory requirements successfully established during the COVID-19 pandemic make mRNA-based vaccination approaches attractive for personalized medical interventions such as precision cancer preventive vaccines. Other innovations in mRNA vaccine platforms include the use of self-amplifying RNA (saRNA) (144–148), circular RNA (circRNA) (149, 150) and modified LNP formulations for mRNA delivery (151–155). While these non-linear mRNA molecule-based vaccines are expected to offer "amplified" expression of encoded proteins *in vivo* requiring lower RNA doses (saRNA) and improved scalability with potentially lower toxicity concerns (circRNA), novel formulations of LNP can be developed to steer the host immune system towards mounting specific immune responses desired for the intended applications (e.g., Th1 *vs.* Th2 immunity) (156). Innovative engineering of RNA molecules and their delivery systems is expected to further help advance the optimization of next-gen RNA vaccine design strategies for precision cancer preventive vaccines for LS and other HCS.

The gold standard of screening for immunogenic recurrent neoantigens, for example for "off the shelf" vaccines, has been to test an individuals' autologous T cells for immune responses to specific neoantigen peptides *in vitro* as measured by interferon gamma production either by ELISPOT or fluorescent activated cell sorting assays. For personalized neoantigen-based therapeutic cancer vaccines, however, because of the large number of potential candidate neoantigens, individualized immunogenicity screening is challenging and time-consuming, which is not ideal due to the urgency for starting vaccinations in patients who have established cancers. Thus, immunizing peptides were selected based on the basis of HLA binding predictions for personalized cancer therapeutic vaccines (41, 42). For off the shelf antigens, one new approach is screening in transgenic mice with human HLA. Currently, these are available for selected alleles including HLA-A2 (157), HLA-A1 (158), and HLA-B7 (159). Importantly, these models have ablated endogenous murine MHC (157). More recently, a series of HLA class I knock-in (KI) mouse strains have been generated (160). In these novel HLA class I transgenic mice, a chimeric HLA class I molecule ($\alpha1/\alpha2$ domain of HLA-A and $\alpha3$ domain of H-2D^b) was covalently linked with 15 aa to human Beta-2-Microglobulin (B2M) and introduced into the endogenous mouse *B2m* locus, resulting in the loss of endogenous mouse MHC class molecules in homozygous KI mice. HLA-restricted, epitope-specific

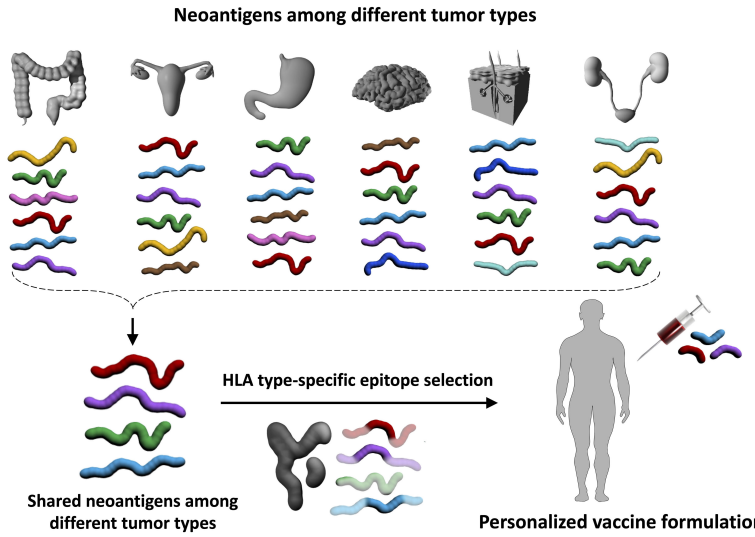


FIGURE 3
Immunopreventive potential of neoantigens shared by tumors in different organs. In addition to the characteristic LS-associated tumors in the colorectum and endometrium, the tumor spectrum in LS also encompasses other organs such as brain, skin, and kidney tumors. It is possible that certain neoantigens are shared across different tumor types (red, purple, green, and aqua blue antigens in the upper panel). Immunopreventive vaccines with these shared neoantigens that are further adjusted by an individual's HLA genotype may allow the development of a personalized, preventive vaccine without organ restriction. Such an approach would be particularly valuable for cancers without screening options. This is illustrated by the example in the lower panel of the figure, where one of the shared neoantigens (green) is not included in the vaccine formulation because this green antigen does not contain neoepitopes that bind to the individual's HLA molecules.

cytotoxic T cells (CTLs) were induced in HLA KI mice upon vaccination (160). These HLA mouse models can be used as a filter towards selecting human common neoantigens as potential vaccine cargo. However, given the diversity of human HLA alleles (161), and the lack of HLA-C mouse models, these models can only be used for frequent HLA alleles.

As with any other agents under development, the demonstration of efficacy is of paramount importance to the successful development of cancer preventive vaccines. If a cancer-free period is used as a primary endpoint for efficacy in cancer prevention studies, however, it will require a larger number of study subjects and long-term follow-up in order to obtain conclusive evidence (162, 163) even in the LS and other cohorts with an increased cancer risk. Therefore, potential surrogate biomarkers, if carefully selected and included in cancer prevention clinical trials, will help delineate clinical correlates of cancer-preventive efficacies. Long-term fortification of the host immune defense against cancer is the ultimate goal of cancer preventive vaccines. Such vaccines must be able to drive and maintain antitumor immune surveillance that can effectively intercept and eliminate emerging tumor precursor cells while avoiding the immune evasion. Because immune biomarkers of cancer preventive vaccines' efficacies have yet to be fully elucidated, multi-pronged research strategies are needed to establish immune correlates of protection, including emerging knowledge from immune biomarker studies conducted in cancer patients (164, 165), which may inform the direction of research. For example, clinically beneficial adaptive antitumor immune responses have been characterized locally in the TME and systemically. Tumor infiltrates in the TME with higher densities of antigen-specific Th1 cells, CTLs, and memory T cells, and lower densities of immunosuppressive T regulatory cells and myeloid derived suppressive cells (MDSC) are generally predictive of better outcomes in cancer patients (164, 166–172). Systemically, immune signature of more favorable responses to ICI immunotherapy has been observed in patients, who at baseline had a diverse TCR repertoire (173), a higher number of $CD8^+$ effector T cells in the periphery and at the tumor margin (174, 175), and a lower level of MDSC (176–178), and had higher levels of TCR repertoire (173, 179), increased levels of $CD127^{low}$ $PD\text{-}1^{low}$ CD4 T cells (180), and peripheral expansion of $CD8^+$ T cells (181–183) at post-treatment. Evaluation of some of these immune biomarkers that are linked to favorable clinical outcomes should be included in preclinical and clinical studies of candidate cancer vaccines, so the immune response profiles can be correlated with *in vivo* antitumor efficacies observed in vaccinated animals and with surrogate biomarkers of efficacies in human study subjects, respectively.

More recently, the roles of tissue-resident memory $CD8^+$ T (T_{RM}) cells have been extensively studied in cancer immunosurveillance. T_{RM} cells are known to function as "pathogen alert" system against invading pathogens for the local organ systems (184–187). Mounting evidence suggests that T_{RM} cells are a critical component of the host immune surveillance and defense mechanisms against developing cancer (184–186, 188–190). A higher number of intratumoral T_{RM} cells is predictive of better overall survival (191–194). Mechanistically, these cells in the TME express immune checkpoint receptors (ICR) and exert antitumor effector functions when ICR are blocked by ICI (188, 189, 195), thus linking the presence of T_{RM} in the TME to more favorable responses to ICI in cancer patients. Furthermore, T_{RM} cells have been shown to recognize neoantigens (192, 193, 196) and can amplify ICI-

mediated antitumor immunity not only by exerting effector functions but also promoting epitope spreading through dendritic cells (193, 196). Preclinical studies have shown T_{RM} cells can be induced by vaccination against tumor neoantigens and that vaccine-induced T_{RM} cells potentiated the host antitumor immunity, rejecting tumor challenge (192, 197, 198). In contrast to clinically beneficial cell-mediated antitumor immune responses widely reported to date, the prognostic value of B cell-mediated humoral immune responses in cancer patients has yet to be fully elucidated (164). Recently, a higher number of B cells in the TME and the presence of intratumoral tertiary lymphoid structures, which include B cell follicles as well as T cells, macrophages, and dendritic cells, have been observed in patients who had better clinical outcomes (164, 199). The role of B-cell mediated immunity for cancer control warrants further investigation.

As discussed earlier, in the first clinical study with trivalent FSP vaccine in patients with LS-associated or non-LS associated MSI-H CRC, all evaluable vaccinated patients showed FSP-specific humoral and predominantly $CD4^+$ T cell responses (48). In the *VcMsh2* LS mouse study discussed earlier, murine rFSP vaccination elicited robust FSP antigen-specific T cell responses ($CD4^+$ and/or $CD8^+$) and humoral immune responses systemically and upregulated intratumoral Th1 signaling pathway more so than Th2. Moreover, intestinal tumors from vaccinated *VcMsh2* mice had significantly elevated levels of $CD4^+$ and $CD8^+$ T cell infiltrates as compared to control tumors (86). These immune response findings are consistent with what has been reported clinically and suggest that rFSP vaccine-induced immune responses were responsible for the observed cancer preventive efficacy *in vivo*. Cancer preventive vaccines, regardless of whether based on commonly shared tumor antigens or personalized neoantigen repertoire (predicted or omics-informed), should be able to at a minimum elicit clinically beneficial antitumor immunity discussed above ideally with long-term memory. To learn and establish immune correlates of protection against cancer, these immune parameters should be included in clinical trials for cancer preventive vaccines as part of investigative biomarker analysis.

In the premalignant and pre-invasive stage setting, there is accumulating evidence to suggest pro-tumorigenic MDSC and immune escape mechanisms mediated through immune checkpoints and immune-suppressive interleukins are already present and contributing to the malignant progression (200–202), which may suppress or hinder adaptive antitumor immune responses to cancer vaccines. Immune profiling of LS polyps has previously demonstrated a significantly increased level of pro-inflammatory and immune checkpoint molecules (203). Cancer vaccine-induced antitumor immunity, therefore, may not be sufficient to effectively prevent or arrest tumorigenic process especially in the setting of intercepting premalignant lesions. According to current knowledge, about two-thirds of LS CRC develop from MMRd crypts, suggesting loss of function of the MMR system as the initiating somatic event (204–206). In such a scenario, the generation of MMRd-triggered FSP likely precedes local immunosuppression, potentially opening a window for vaccine-mediated interception. However, the differential effectiveness of rFSP vaccines for the prevention of LS CRC triggered by MMRd *vs*. those resulting from MMR-proficient adenomas and subsequent MMR inactivation needs to be evaluated in future studies.

The addition of immunomodulatory agents to cancer vaccines may be warranted to induce more robust adaptive immune responses for LS and other high-risk cohorts. For example, chemo preventive effects of NSAID (aspirin and naproxen) have been extensively studied in LS patients (9, 87, 88). In addition to directly reducing the level of pro-tumorigenic prostaglandin E2 (88), naproxen has been shown to potentiate antitumor immune responses by rFSP vaccination in the *VcMsh2* mouse study (86) and boosted immune surveillance in LS patients (88). In preclinical models of CRC tumorigenesis, naproxen administration has been shown to decrease the expression of PD-L1 in colon tumors and increase the density of $CD8^+$ TILs (207). Since the efficacy of ICI has already been demonstrated in LS and MSI-high cancer patients, ICI-based treatment is being considered for immunointerception in the premalignant setting in LS cohort (208). There are other classes of immunomodulatory agents that can be potentially used to boost the host immune responses to cancer-preventive and interceptive vaccines (209–212). Feasibility, efficacy, and safety of combination of cancer preventive vaccines and these newer immunomodulatory agents should be explored especially for LS and high-risk cohorts through preclinical and clinical research.

Concluding remarks

The first clinical study with FSP neoantigen-based cancer vaccine (NCT01461148) was launched more than a decade ago in MMR-deficient colon cancer patients (48). This seminal study, which was built on the culmination of many years of extensive research on LS tumor molecular biology and endogenous immunity led by the same group, Kloor, von Knebel Doeberitz, and their colleagues, has dramatically changed the landscape of neoantigen-based cancer vaccine research. Over the last decade, there has been an explosion of research on tumorigenesis and genetic triggers, tumor immune surveillance, immune checkpoint mechanisms that can unleash antitumor immunity, contexture of tumor-immune microenvironment, and dynamic interplay between evolving tumors and immune defense, all of which are generating the consensus that better cancer control and favorable outcomes are achievable if tumorigenesis is intercepted earlier than later. Together with technological advances in tumor genomic landscape profiling, cancer vaccinology, and innovative immunomodulatory agents, precision cancer prevention and interception for LS carriers is within the reach. There are, however, remaining questions that must be addressed. For example, even if technical challenges of personalized FSP vaccine production can be overcome, can personalized neoantigen-based precision cancer vaccines lead to more efficacious and long-term immune protection than shared FSP neoantigen-based vaccines in LS carriers? To remain cancer free, how long do LS carriers need to maintain antitumor immune memory? Does the combination of

immunomodulatory agents help sustain the durability of immune protection in LS carriers? There are translational barriers that also need to be overcome before the true benefit of precision cancer preventive vaccines are realized for LS carriers. The preclinical research field will greatly benefit from better preclinical models that can more closely mimic human LS tumorigenesis and human immune system. Newer generation of humanized preclinical models may help bridge the inter-species knowledge gap that has been a major obstacle in translational research for LS and other cancer vaccines. Lastly, as next-generation novel surrogate markers emerge from preclinical and clinical studies in the next decade, regulatory approval pathways will have to be reviewed and improved for scientific harmonization without delay. The success of FSP neoantigen-based cancer vaccines for LS cancer prevention will hopefully demonstrate the potential marketability of cancer preventive vaccines in the next decade, which will bring an increasing interest from the private sector and can lead to the partnership opportunities between academia, government, and industry for the betterment of quality of life for LS and other high-risk populations.

Author contributions

SS, AA, DK, SL, and MK wrote this review. LB, JG, and MKD critically reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by NIH grants U54 CA 272688 (DK, JG, MKD, SL, MK), U01 CA233056 (JG, MKD, SL, MK), and R01 HL157174 (DK), grants from German Cancer Aid (Deutsche Krebshilfe, 70113455, 70113890), German Research Foundation (Deutsche Forschungsgemeinschaft, GE 592-9/1), and Donations against Cancer, NCT Heidelberg, and contracts from NCI PREVENT program (HHSN2612015000391).

Acknowledgments

The authors thank Drs. Olivera (Olja) J. Finn and Mary L. (Nora) Disis for helpful discussions, Drs. Asad Umar and Robert Shoemaker for supporting the PREVENT program, and all patients and patient organizations (SemiColon, Germany) involved in the research on vaccines against Lynch syndrome tumors.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Author disclaimer

The opinions expressed by the authors are their own and this material should not be interpreted as representing the official viewpoint of the US Department of Health and Human Services, the National Institutes of Health, or the National Cancer Institute.

References

1. Williams PA, Zaidi SK, Sengupta R. AACR cancer progress report 2022: Decoding cancer complexity, integrating science, and transforming patient outcomes. *Clin Cancer Res* (2022) 28(19):4178–9. doi: 10.1158/1078-0432.CCR-22-2588

2. Tomasetti C, Vogelstein B. Cancer etiology. variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science* (2015) 347(6217):78–81. doi: 10.1126/science.1260825

3. Tsaousis GN, Papadopoulou E, Apessos A, Agiannitopoulos K, Pepe G, Kampouri S, et al. Analysis of hereditary cancer syndromes by using a panel of genes: novel and multiple pathogenic mutations. *BMC Cancer* (2019) 19(1):535. doi: 10.1186/s12885-019-5756-4

4. Offit K, Tkachuk KA, Stadler ZK, Walsh MF, Diaz-Zabala H, Levin JD, et al. Cascading after peridiagnostic cancer genetic testing: An alternative to population-based screening. *J Clin Oncol* (2020) 38(13):1398–408. doi: 10.1200/JCO.19.02010

5. Yurgelun MB, Uno H, Furniss CS, Ukaegbu C, Horiguchi M, Yussuf A, et al. Development and validation of the PREMMplus model for multigene hereditary cancer risk assessment. *J Clin Oncol* (2022), 40(35):4083–94. doi: 10.1200/JCO.22.00120

6. Walsh MF, Cadoo K, Salo-Mullen EE, Dubard-Gault M, Stadler ZK, Offit K. Genetic factors: Hereditary cancer predisposition syndromes. In: Niederhuber JE, Armitage JO, Doroshow JH, Kastan MB, Tepper JE, editors. *Abeloff's clinical oncology, 6th ed*. Philadelphia, PA: Elsevier (2020). p. 180–208.e11.

7. Blackburn EH. Cancer interception. *Cancer Prev Res (Phila)* (2011) 4(6):787–92. doi: 10.1158/1940-6207.CAPR-11-0195

8. Crosby D, Bhatia S, Brindle KM, Coussens LM, Dive C, Emberton M, et al. Early detection of cancer. *Science* (2022) 375(6586):eaay9040. doi: 10.1126/science.aay9040

9. Burn J, Sheth H, Elliott F, Reed L, Macrae F, Mecklin JP, 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(10240):1855–63. doi: 10.1016/S0140-6736(20)30366-4

10. Muhoza P, Danovaro-Holliday MC, Diallo MS, Murphy P, Sodha SV, Requejo JH, et al. Routine vaccination coverage - worldwide, 2020. *MMWR Morb Mortal Wkly Rep* (2021) 70(43):1495–500. doi: 10.15585/mmwr.mm7043a1

11. Tregoning JS, Flight KE, Higham SL, Wang Z, Pierce BF. Progress of the COVID-19 vaccine effort: viruses, vaccines and variants versus efficacy, effectiveness and escape. *Nat Rev Immunol* (2021) 21(10):626–36. doi: 10.1038/s41577-021-00592-1

12. Corey L, Mascola JR, Fauci AS, Collins FS. A strategic approach to COVID-19 vaccine R&D. *Science* (2020) 368(6494):948–50. doi: 10.1126/science.abc5312

13. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol* (2021) 21(2):83–100. doi: 10.1038/s41577-020-00479-7

14. Finn OJ. Cancer vaccines: between the idea and the reality. *Nat Rev Immunol* (2003) 3(8):630–41. doi: 10.1038/nri1150

15. Graham JB, Graham RM. The effect of vaccine on cancer patients. *Surg Gynecol Obstet* (1959) 109(2):131–8. doi: 10.1097/00006534-195911000-00016

16. Burnet M. Cancer: a biological approach. III. viruses associated with neoplastic conditions. IV. practical applications. *Br Med J* (1957) 1(5023):841–7. doi: 10.1136/bmj.1.5023.841

17. Dobosz P, Dzieciatkowski T. The intriguing history of cancer immunotherapy. *Front Immunol* (2019) 10:2965. doi: 10.3389/fimmu.2019.02965

18. Schlom J, Hodge JW, Palena C, Tsang KY, Jochems C, Greiner JW, et al. Therapeutic cancer vaccines. *Adv Cancer Res* (2014) 121:67–124. doi: 10.1016/B978-0-12-800249-0.00002-0

19. Barnd DL, Lan MS, Metzgar RS, Finn OJ. Specific, major histocompatibility complex-unrestricted recognition of tumor-associated mucins by human cytotoxic T cells. *Proc Natl Acad Sci U.S.A.* (1989) 86(18):7159–63. doi: 10.1073/pnas.86.18.7159

20. Finn OJ. Premalignant lesions as targets for cancer vaccines. *J Exp Med* (2003) 198(11):1623–6. doi: 10.1084/jem.20031787

21. Finn OJ. Vaccines for cancer prevention: a practical and feasible approach to the cancer epidemic. *Cancer Immunol Res* (2014) 2(8):708–13. doi: 10.1158/2326-6066.CIR-14-0110

22. Finn OJ. A believer's overview of cancer immunosurveillance and immunotherapy. *J Immunol* (2018) 200(2):385–91. doi: 10.4049/jimmunol.1701302

23. Disis ML, Gooley TA, Rinn K, Davis D, Piepkorn M, Cheever MA, et al. Generation of T-cell immunity to the HER-2/neu protein after active immunization with HER-2/neu peptide-based vaccines. *J Clin Oncol* (2002) 20(11):2624–32. doi: 10.1200/JCO.2002.06.171

24. Knutson KL, Schiffman K, Cheever MA, Disis ML. Immunization of cancer patients with a HER-2/neu, HLA-A2 peptide, p369-377, results in short-lived peptide-specific immunity. *Clin Cancer Res* (2002) 8(5):1014–8.

25. Lollini PL, Cavallo F, Nanni P, Forni G. Vaccines for tumour prevention. *Nat Rev Cancer* (2006) 6(3):204–16. doi: 10.1038/nrc1815

26. Salazar LG, Fikes J, Southwood S, Ishioka G, Knutson KL, Gooley TA, et al. Immunization of cancer patients with HER-2/neu-derived peptides demonstrating high-affinity binding to multiple class II alleles. *Clin Cancer Res* (2003) 9(15):5559–65.

27. Park KH, Gad E, Goodell V, Dang Y, Wild T, Higgins D, et al. Insulin-like growth factor-binding protein-2 is a target for the immunomodulation of breast cancer. *Cancer Res* (2008) 68(20):8400–9. doi: 10.1158/0008-5472.CAN-07-5891

28. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. *Sci Transl Med* (2016) 8(328):328rv4. doi: 10.1126/scitranslmed.aad7118

29. Wei SC, Duffy CR, Allison JP. Fundamental mechanisms of immune checkpoint blockade therapy. *Cancer Discovery* (2018) 8(9):1069–86. doi: 10.1158/2159-8290.CD-18-0367

30. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science* (2015) 348(6230):69–74. doi: 10.1126/science.aaa4971

31. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* (2015) 348(6230):124–8. doi: 10.1126/science.aaa1348

32. Linnemann C, van Buuren MM, Bies L, Verdegaal EM, Schotte R, Calis JJ, et al. High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma. *Nat Med* (2015) 21(1):81–5. doi: 10.1038/nm.3773

33. Spira A, Disis ML, Schiller JT, Vilar E, Rebbeck TR, Bejar R, et al. Leveraging premalignant biology for immune-based cancer prevention. *Proc Natl Acad Sci U S A* (2016) 113(39):10750–8. doi: 10.1073/pnas.1608077113

34. Chen B, Scurrah CR, McKinley ET, Simmons AJ, Ramirez-Solano MA, Zhu X, et al. Differential pre-malignant programs and microenvironment chart distinct paths to malignancy in human colorectal polyps. *Cell* (2021) 184(26):6262–80.e26. doi: 10.1016/j.cell.2021.11.031

35. Rebbeck TR, Burns-White K, Chan AT, Emmons K, Freedman M, Hunter DJ, et al. Precision prevention and early detection of cancer: Fundamental principles. *Cancer Discovery* (2018) 8(7):803–11. doi: 10.1158/2159-8290.CD-17-1415

36. Lin MJ, Svensson-Arvelund J, Lubitz GS, Marabelle A, Melero I, Brown BD, et al. Cancer vaccines: the next immunotherapy frontier. *Nat Cancer* (2022) 3(8):911–26. doi: 10.1038/s43018-022-00418-6

37. Garraway LA, Verweij J, Ballman KV. Precision oncology: an overview. *J Clin Oncol* (2013) 31(15):1803–5. doi: 10.1200/JCO.2013.49.4799

38. Schumacher TN, Scheper W, Kvistborg P. Cancer neoantigens. *Annu Rev Immunol* (2019) 37:173–200. doi: 10.1146/annurev-immunol-042617-053402

39. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, et al. Patterns of somatic mutation in human cancer genomes. *Nature* (2007) 446(7132):153–8. doi: 10.1038/nature05610

40. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LAJr., Kinzler KW. Cancer genome landscapes. *Science* (2013) 339(6127):1546–58. doi: 10.1126/science.1235122

41. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* (2017) 547(7662):217–21. doi: 10.1038/nature22991

42. Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, Lower M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* (2017) 547(7662):222–6. doi: 10.1038/nature23003

43. Hu Z, Leet DE, Allesoe RL, Oliveira G, Li S, Luoma AM, et al. Personal neoantigen vaccines induce persistent memory T cell responses and epitope spreading in patients with melanoma. *Nat Med* (2021) 27(3):515–25. doi: 10.1038/s41591-020-01206-4

44. von Knebel Doeberitz M, Kloor M. Towards a vaccine to prevent cancer in lynch syndrome patients. *Fam Cancer* (2013) 12(2):307–12. doi: 10.1007/s10689-013-9662-7

45. Kloor M, von Knebel Doeberitz M. The immune biology of microsatellite-unstable cancer. *Trends Cancer* (2016) 2(3):121–33. doi: 10.1016/j.trecan.2016.02.004

46. Woerner SM, Kloor M, Mueller A, Rueschoff J, Friedrichs N, Buettner R, et al. Microsatellite instability of selective target genes in HNPCC-associated colon adenomas. *Oncogene* (2005) 24(15):2525–35. doi: 10.1038/sj.onc.1208456

47. Schwitalle Y, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, et al. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. *Gastroenterology* (2008) 134(4):988–97. doi: 10.1053/j.gastro.2008.01.015

48. Kloor M, Reuschenbach M, Pauligk C, Karbach J, Rafiyan MR, Al-Batran SE, et al. A frameshift peptide neoantigen-based vaccine for mismatch repair-deficient cancers: A phase I/IIa clinical trial. *Clin Cancer Res* (2020) 26(17):4503–10. doi: 10.1158/1078-0432.CCR-19-3517

49. Tabori U, Hansford JR, Achatz MI, Kratz CP, Plon SE, Frebourg T, et al. Clinical management and tumor surveillance recommendations of inherited mismatch repair deficiency in childhood. *Clin Cancer Res* (2017) 23(11):e32–e7. doi: 10.1158/1078-0432.CCR-17-0574

50. Chung J, Negm L, Bianchi V, Stengs L, Das A, Liu ZA, et al. Genomic microsatellite signatures identify germline mismatch repair deficiency and risk of cancer onset. *J Clin Oncol* (2022) 41(4):766–77. doi: 10.1136/jmedgenet-2014-102284

51. Wimmer K, Kratz CP, Vasen HF, Caron O, Colas C, Entz-Werle N, et al. Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'care for CMMRD' (C4CMMRD). *J Med Genet* (2014) 51(6):355–65. doi: 10.1136/jmedgenet-2014-102284

52. Das A, Sudhaman S, Morgenstern D, Coblentz A, Chung J, Stone SC, et al. Genomic predictors of response to PD-1 inhibition in children with germline DNA replication repair deficiency. *Nat Med* (2022) 28(1):125–35. doi: 10.1038/s41591-021-01581-6

53. Carethers JM, Stoffel EM. Lynch syndrome and lynch syndrome mimics: The growing complex landscape of hereditary colon cancer. *World J Gastroenterol* (2015) 21(31):9253–61. doi: 10.3748/wjg.v21.i31.9253

54. Stadler ZK. Diagnosis and management of DNA mismatch repair-deficient colorectal cancer. *Hematol Oncol Clin North Am* (2015) 29(1):29–41. doi: 10.1016/j.hoc.2014.09.008

55. Rumilla K, Schowalter KV, Lindor NM, Thomas BC, Mensink KA, Gallinger S, et al. Frequency of deletions of EPCAM (TACSTD1) in MSH2-associated lynch syndrome cases. *J Mol Diagn* (2011) 13(1):93–9. doi: 10.1016/j.jmoldx.2010.11.011

56. Haraldsdottir S, Rafnar T, Frankel WL, Einarsdottir S, Sigurdsson A, Hampel H, et al. Comprehensive population-wide analysis of lynch syndrome in Iceland reveals founder mutations in MSH6 and PMS2. *Nat Commun* (2017) 8:14755. doi: 10.1038/ncomms14755

57. Olkinuora A, Nieminen TT, Martensson E, Rohlin A, Ristimaki A, Koskenvuo L, et al. Biallelic germline nonsense variant of MLH3 underlies polyposis predisposition. *Genet Med* (2019) 21(8):1868–73. doi: 10.1038/s41436-018-0405-x

58. Lipkin SM, Wang V, Jacoby R, Banerjee-Basu S, Baxevanis AD, Lynch HT, et al. MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. *Nat Genet* (2000) 24(1):27–35. doi: 10.1038/71643

59. Lipkin SM, Wang V, Stoler DL, Anderson GR, Kirsch I, Hadley D, et al. Germline and somatic mutation analyses in the DNA mismatch repair gene MLH3: Evidence for somatic mutation in colorectal cancers. *Hum Mutat* (2001) 17(5):389–96. doi: 10.1002/humu.1114

60. Adam R, Spier I, Zhao B, Kloth M, Marquez J, Hinrichsen I, et al. Exome sequencing identifies biallelic MSH3 germline mutations as a recessive subtype of colorectal adenomatous polyposis. *Am J Hum Genet* (2016) 99(2):337–51. doi: 10.1016/j.ajhg.2016.06.015

61. Yurgelun MB, Hampel H. Recent advances in lynch syndrome: Diagnosis, treatment, and cancer prevention. *Am Soc Clin Oncol Educ Book* (2018) 38:101–9. doi: 10.1200/EDBK_208341

62. Muller C, Lee SM, Barge W, Siddique SM, Berera S, Wideroff G, et al. Low referral rate for genetic testing in racially and ethnically diverse patients despite universal colorectal cancer screening. *Clin Gastroenterol Hepatol* (2018) 16(12):1911–8.e2. doi: 10.1016/j.cgh.2018.08.038

63. Muller C, Matthews L, Kupfer SS, Weiss JM. Effective identification of lynch syndrome in gastroenterology practice. *Curr Treat Options Gastroenterol* (2019) 17(4):666–80. doi: 10.1007/s11938-019-00261-2

64. Faust N, Muller C, Prenner J, Lee SM, Kupfer SS. Low rates of genetic counseling and testing in individuals at risk for lynch syndrome reported in the national health interview survey. *Gastroenterology* (2020) 158(4):1159–61. doi: 10.1053/j.gastro.2019.11.297

65. Muller C, Nielsen SM, Hatchell KE, Yang S, Michalski ST, Hamlington B, et al. Underdiagnosis of hereditary colorectal cancers among Medicare patients: Genetic testing criteria for lynch syndrome miss the mark. *JCO Precis Oncol* (2021) 5:1103–11. doi: 10.1200/PO.21.00132

66. Bellis MA, Hughes K, Hughes S, Ashton JR. Measuring paternal discrepancy and its public health consequences. *J Epidemiol Community Health* (2005) 59(9):749–54. doi: 10.1136/jech.2005.036517

67. Greeff JM, Erasmus JC. Three hundred years of low non-paternity in a human population. *Heredity (Edinb)* (2015) 115(5):396–404. doi: 10.1038/hdy.2015.36

68. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Screening for the lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med* (2005) 352(18):1851–60. doi: 10.1056/NEJMoa043146

69. Mills AM, Longacre TA. Lynch syndrome screening in the gynecologic tract: Current state of the art. *Am J Surg Pathol* (2016) 40(4):e35–44. doi: 10.1097/PAS.0000000000000608

70. Seppala TT, Latchford A, Negoi I, Sampaio Soares A, Jimenez-Rodriguez R, Sanchez-Guillen L, et al. European Guidelines from the EHTG and ESCP for lynch syndrome: an updated third edition of the mallorca guidelines based on gene and gender. *Br J Surg* (2021) 108(5):484–98. doi: 10.1002/bjs.11902

71. Snowsill T, Huxley N, Hoyle M, Jones-Hughes T, Coelho H, Cooper C, et al. A model-based assessment of the cost-utility of strategies to identify lynch syndrome in early-onset colorectal cancer patients. *BMC Cancer* (2015) 15:313. doi: 10.1186/s12885-015-1254-5

72. Bernstedt SW, Bjork J, Fritzell K, Spigelman AD, Bjorck E, Backman AS. Room for improvement: One third of lynch syndrome patients presenting for genetic testing in a highly specialised centre in Stockholm already have cancer. *Hered Cancer Clin Pract* (2021) 19(1):18. doi: 10.1186/s13053-021-00171-4

73. Vasen HF, Moslein G, Alonso A, Aretz S, Bernstein I, Bertario L, et al. Recommendations to improve identification of hereditary and familial colorectal cancer in Europe. *Fam Cancer* (2010) 9(2):109–15. doi: 10.1007/s10689-009-9291-3

74. Singh H, Schiesser R, Anand G, Richardson PA, El-Serag HB. Underdiagnosis of lynch syndrome involves more than family history criteria. *Clin Gastroenterol Hepatol* (2010) 8(6):523–9. doi: 10.1016/j.cgh.2010.03.010

75. Kahn RM, Ahsan MD, Chapman-Davis E, Holcomb K, Nitecki R, Rauh-Hain JA, et al. Barriers to completion of cascade genetic testing: how can we improve the uptake of testing for hereditary breast and ovarian cancer syndrome? *Fam Cancer* (2022). doi: 10.1007/s10689-022-00316-x

76. Frey MK, Ahsan MD, Bergeron H, Lin J, Li X, Fowlkes RK, et al. Cascade testing for hereditary cancer syndromes: Should we move toward direct relative contact? a systematic review and meta-analysis. *J Clin Oncol* (2022) 40(35):4129–43. doi: 10.1200/JCO.22.00303

77. Frey MK, Kahn RM, Chapman-Davis E, Tubito F, Pires M, Christos P, et al. Prospective feasibility trial of a novel strategy of facilitated cascade genetic testing using telephone counseling. *J Clin Oncol* (2020) 38(13):1389–97. doi: 10.1200/JCO.19.02005

78. Slavin TP, Banks KC, Chudova D, Oxnard GR, Odegaard JI, Nagy RJ, et al. Identification of incidental germline mutations in patients with advanced solid tumors who underwent cell-free circulating tumor DNA sequencing. *J Clin Oncol* (2018) 36(35):3459–65. doi: 10.1200/JCO.18.00328

79. Zviran A, Schulman RC, Shah M, Hill STK, Deochand S, Khamnei CC, et al. Genome-wide cell-free DNA mutational integration enables ultra-sensitive cancer monitoring. *Nat Med* (2020) 26(7):1114–24. doi: 10.1038/s41591-020-0915-3

80. Kather JN, Pearson AT, Halama N, Jager D, Krause J, Loosen SH, et al. Deep learning can predict microsatellite instability directly from histology in gastrointestinal cancer. *Nat Med* (2019) 25(7):1054–6. doi: 10.1038/s41591-019-0462-y

81. Hildebrand LA, Pierce CJ, Dennis M, Paracha M, Maoz A. Artificial intelligence for histology-based detection of microsatellite instability and prediction of response to immunotherapy in colorectal cancer. *Cancers (Basel)* (2021) 13(3):391. doi: 10.3390/cancers13030391

82. Rousseau B, Foote MB, Maron SB, Diplas BH, Lu S, Argiles G, et al. The spectrum of benefit from checkpoint blockade in hypermutated tumors. *N Engl J Med* (2021) 384(12):1168–70. doi: 10.1056/NEJMc2031965

83. Dominguez-Valentin M, Sampson JR, Seppala TT, Ten Broeke SW, Plazzer JP, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the prospective lynch syndrome database. *Genet Med* (2020) 22(1):15–25. doi: 10.1038/s41436-019-0596-9

84. Woerner SM, Yuan YP, Benner A, Korff S, von Knebel Doeberitz M, Bork P. SelTarbase, a database of human mononucleotide-microsatellite mutations and their potential impact to tumorigenesis and immunology. *Nucleic Acids Res* (2010) 38(Database issue):D682–9. doi: 10.1093/nar/gkp839

85. Kucherlapati MH, Lee K, Nguyen AA, Clark AB, Hou HJr., Rosulek A, et al. An Msh2 conditional knockout mouse for studying intestinal cancer and testing anticancer agents. *Gastroenterology* (2010) 138(3):993–1002 e1. doi: 10.1053/j.gastro.2009.11.009

86. Gebert J, Gelincik O, Oezcan-Wahlbrink M, Marshall JD, Hernandez-Sanchez A, Urban K, et al. Recurrent frameshift neoantigen vaccine elicits protective immunity with reduced tumor burden and improved overall survival in a lynch syndrome mouse model. *Gastroenterology* (2021) 161(4):1288–302.e13. doi: 10.1053/j.gastro.2021.06.073

87. Burn J, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. *Lancet* (2011) 378(9809):2081–7. doi: 10.1016/S0140-6736(11)61049-0

88. Reyes-Uribe L, Wu W, Gelincik O, Bommi PV, Francisco-Cruz A, Solis LM, et al. Naproxen chemoprevention promotes immune activation in lynch syndrome colorectal mucosa. *Gut* (2021) 70(3):555–66. doi: 10.1136/gutjnl-2020-320946

89. Wang D, Dubois RN. Eicosanoids and cancer. *Nat Rev Cancer* (2010) 10(3):181–93. doi: 10.1038/nrc2809

90. Wang D, Dubois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. *Oncogene* (2010) 29(6):781–8. doi: 10.1038/onc.2009.421

91. Ait Ouakrim D, Dashti SG, Chau R, Buchanan DD, Clendenning M, Rosty C, et al. Aspirin, ibuprofen, and the risk of colorectal cancer in lynch syndrome. *J Natl Cancer Inst* (2015) 107(9):djv170. doi: 10.1093/jnci/djv170

92. Leoni G, D'Alise AM, Cotugno G, Langone F, Garzia I, De Lucia M, et al. A genetic vaccine encoding shared cancer neoantigens to treat tumors with microsatellite instability. *Cancer Res* (2020) 80(18):3972–82. doi: 10.1158/0008-5472.CAN-20-1072

93. Toes RE, Ossendorp F, Offringa R, Melief CJ. CD4 T cells and their role in antitumor immune responses. *J Exp Med* (1999) 189(5):753–6. doi: 10.1084/jem.189.5.753

94. Axelrod ML, Cook RS, Johnson DB, Balko JM. Biological consequences of MHC-II expression by tumor cells in cancer. *Clin Cancer Res* (2019) 25(8):2392–402. doi: 10.1158/1078-0432.CCR-18-3200

95. Falk K, Rotzschke O, Stevanovic S, Jung G, Rammensee HG. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* (1991) 351(6324):290–6. doi: 10.1038/351290a0

96. Jurtz V, Paul S, Andreatta M, Marcatili P, Peters B, Nielsen M. NetMHCpan-4.0: Improved peptide-MHC class I interaction predictions integrating eluted ligand and peptide binding affinity data. *J Immunol* (2017) 199(9):3360–8. doi: 10.4049/jimmunol.1700893

97. Langton DJ, Bourke SC, Lie BA, Reiff G, Natu S, Darlay R, et al. The influence of HLA genotype on the severity of COVID-19 infection. *HLA* (2021) 98(1):14–22. doi: 10.1111/tan.14284

98. Crocchiolo R, Gallina AM, Pani A, Campisi D, Cento V, Sacchi N, et al. Polymorphism of the HLA system and weak antibody response to BNT162b2 mRNA vaccine. *HLA* (2022) 99(3):183–91. doi: 10.1111/tan.14546

99. Francis JM, Leistritz-Edwards D, Dunn A, Tarr C, Lehman J, Dempsey C, et al. Allelic variation in class I HLA determines CD8(+) T cell repertoire shape and cross-reactive memory responses to SARS-CoV-2. *Sci Immunol* (2022) 7(67):eabk3070. doi: 10.1126/sciimmunol.abk3070

100. Ahadova A, Witt J, Haupt S, Gallon R, Huneburg R, Nattermann J, et al. Is HLA type a possible cancer risk modifier in lynch syndrome? *Int J Cancer* (2023) 152(10):2024–31. doi: 10.1002/ijc.34312

101. Ballhausen A, Przybilla MJ, Jendrusch M, Haupt S, Pfaffendorf E, Seidler F, et al. The shared frameshift mutation landscape of microsatellite-unstable cancers suggests immunoediting during tumor evolution. *Nat Commun* (2020) 11(1):4740. doi: 10.1038/s41467-020-18514-5

102. Bohaumilitzky L, Kluck K, Huneburg R, Gallon R, Nattermann J, Kirchner M, et al. The different immune profiles of normal colonic mucosa in cancer-free lynch syndrome carriers and lynch syndrome colorectal cancer patients. *Gastroenterology* (2022) 162(3):907–19.e10. doi: 10.1053/j.gastro.2021.11.029

103. Witt J, Haupt S, Ahadova A, Bohaumilitzky L, Fuchs V, Ballhausen A, et al. A simple approach for detecting HLA-A*02 alleles in archival formalin-fixed paraffin-embedded tissue samples and an application example for studying cancer immunoediting. *HLA* (2023) 101(1):24–33. doi: 10.1111/tan.14846

104. Nelde A, Maringer Y, Bilich T, Salih HR, Roerden M, Heitmann JS, et al. Immunopeptidomics-guided warehouse design for peptide-based immunotherapy in chronic lymphocytic leukemia. *Front Immunol* (2021) 12:705974. doi: 10.3389/fimmu.2021.705974

105. Nelde A, Rammensee HG, Walz JS. The peptide vaccine of the future. *Mol Cell Proteomics* (2021) 20:100022. doi: 10.1074/mcp.R120.002309

106. McGranahan N, Rosenthal R, Hiley CT, Rowan AJ, Watkins TBK, Wilson GA, et al. Allele-specific HLA loss and immune escape in lung cancer evolution. *Cell* (2017) 171(6):1259–71.e11. doi: 10.1016/j.cell.2017.10.001

107. McGranahan N, Swanton C. Cancer evolution constrained by the immune microenvironment. *Cell* (2017) 170(5):825–7. doi: 10.1016/j.cell.2017.08.012

108. McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: Past, present, and the future. *Cell* (2017) 168(4):613–28. doi: 10.1016/j.cell.2017.01.018

109. Laughney AM, Hu J, Campbell NR, Bakhoum SF, Setty M, Lavallee VP, et al. Regenerative lineages and immune-mediated pruning in lung cancer metastasis. *Nat Med* (2020) 26(2):259–69. doi: 10.1038/s41591-019-0750-6

110. Anagnostou V, Smith KN, Forde PM, Niknafs N, Bhattacharya R, White J, et al. Evolution of neoantigen landscape during immune checkpoint blockade in non-small cell lung cancer. *Cancer Discovery* (2017) 7(3):264–76. doi: 10.1158/2159-8290.CD-16-0828

111. Kloor M, Huth C, Voigt AY, Benner A, Schirmacher P, von Knebel Doeberitz M, et al. Prevalence of mismatch repair-deficient crypt foci in lynch syndrome: a pathological study. *Lancet Oncol* (2012) 13(6):598–606. doi: 10.1016/S1470-2045(12)70109-2

112. Garraway LA, Lander ES. Lessons from the cancer genome. *Cell* (2013) 153(1):17–37. doi: 10.1016/j.cell.2013.03.002

113. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* (2016) 351(6280):1463–9. doi: 10.1126/science.aaf1490

114. Tran E, Ahmadzadeh M, Lu YC, Gros A, Turcotte S, Robbins PF, et al. Immunogenicity of somatic mutations in human gastrointestinal cancers. *Science* (2015) 350(6266):1387–90. doi: 10.1126/science.aad1253

115. Hilf N, Kuttruff-Coqui S, Frenzel K, Bukur V, Stevanovic S, Gouttefangeas C, et al. Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature* (2019) 565(7738):240–5. doi: 10.1038/s41586-018-0810-y

116. Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, et al. Neoantigen vaccine generates intratumoral T cell responses in phase ib glioblastoma trial. *Nature* (2019) 565(7738):234–9. doi: 10.1038/s41586-018-0792-9

117. Ott PA, Hu-Lieskovan S, Chmielowski B, Govindan R, Naing A, Bhardwaj N, et al. A phase ib trial of personalized neoantigen therapy plus anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer. *Cell* (2020) 183(2):347–62.e24. doi: 10.1016/j.cell.2020.08.053

118. Tran E, Robbins PF, Lu YC, Prickett TD, Gartner JJ, Jia L, et al. T-Cell transfer therapy targeting mutant KRAS in cancer. *N Engl J Med* (2016) 375(23):2255–62. doi: 10.1056/NEJMoa1609279

119. Malekzadeh P, Pasetto A, Robbins PF, Parkhurst MR, Paria BC, Jia L, et al. Neoantigen screening identifies broad TP53 mutant immunogenicity in patients with epithelial cancers. *J Clin Invest* (2019) 129(3):1109–14. doi: 10.1172/JCI123791

120. Hsiue EH, Wright KM, Douglass J, Hwang MS, Mog BJ, Pearlman AH, et al. Targeting a neoantigen derived from a common TP53 mutation. *Science* (2021) 371(6533):eabc8697. doi: 10.1126/science.abc8697

121. Veatch JR, Lee SM, Fitzgibbon M, Chow IT, Jesernig B, Schmitt T, et al. Tumor-infiltrating BRAFV600E-specific CD4+ T cells correlated with complete clinical response in melanoma. *J Clin Invest* (2018) 128(4):1563–8. doi: 10.1172/JCI98689

122. Castle JC, Uduman M, Pabla S, Stein RB, Buell JS. Mutation-derived neoantigens for cancer immunotherapy. *Front Immunol* (2019) 10:1856. doi: 10.3389/fimmu.2019.01856

123. Li F, Deng L, Jackson KR, Talukder AH, Katailiha AS, Bradley SD, et al. Neoantigen vaccination induces clinical and immunologic responses in non-small cell lung cancer patients harboring EGFR mutations. *J Immunother Cancer* (2021) 9(7):e002531. doi: 10.1136/jitc-2021-002531corr1

124. Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. *J Clin Oncol* (2010) 28(31):4722–9. doi: 10.1200/JCO.2010.28.6963

125. Dupain C, Gracia C, Harttrampf AC, Riviere J, Geoerger B, Massaad-Massade L. Newly identified LMO3-BORCS5 fusion oncogene in Ewing sarcoma at relapse is a driver of tumor progression. *Oncogene* (2019) 38(47):7200–15. doi: 10.1038/s41388-019-0914-3

126. Yang W, Lee KW, Srivastava RM, Kuo F, Krishna C, Chowell D, et al. Immunogenic neoantigens derived from gene fusions stimulate T cell responses. *Nat Med* (2019) 25(5):767–75. doi: 10.1038/s41591-019-0434-2

127. Lin HH, Ray S, Tongchusak S, Reinherz EL, Brusic V. Evaluation of MHC class I peptide binding prediction servers: applications for vaccine research. *BMC Immunol* (2008) 9:8. doi: 10.1186/1471-2172-9-8

128. Mei S, Li F, Leier A, Marquez-Lago TT, Giam K, Croft NP, et al. A comprehensive review and performance evaluation of bioinformatics tools for HLA class I peptide-binding prediction. *Brief Bioinform* (2020) 21(4):1119–35. doi: 10.1093/bib/bbz051

129. Sarkizova S, Klaeger S, Le PM, Li LW, Oliveira G, Keshishian H, et al. A large peptidome dataset improves HLA class I epitope prediction across most of the human population. *Nat Biotechnol* (2020) 38(2):199–209. doi: 10.1038/s41587-019-0322-9

130. Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, et al. The immune epitope database (IEDB): 2018 update. *Nucleic Acids Res* (2019) 47(D1):D339–D43. doi: 10.1093/nar/gky1006

131. Zhang GL, Lin HH, Keskin DB, Reinherz EL, Brusic V. Dana-Farber Repository for machine learning in immunology. *J Immunol Methods* (2011) 374(1-2):18–25. doi: 10.1016/j.jim.2011.07.007

132. Abelin JG, Keskin DB, Sarkizova S, Hartigan CR, Zhang W, Sidney J, et al. Mass spectrometry profiling of HLA-associated peptidomes in mono-allelic cells enables more accurate epitope prediction. *Immunity* (2017) 46(2):315–26. doi: 10.1016/j.immuni.2017.02.007

133. Keskin DB, Reinhold BB, Zhang GL, Ivanov AR, Karger BL, Reinherz EL. Physical detection of influenza a epitopes identifies a stealth subset on human lung epithelium evading natural CD8 immunity. *Proc Natl Acad Sci U.S.A.* (2015) 112(7):2151–6. doi: 10.1073/pnas.1423482112

134. Rasmussen M, Fenoy E, Harndahl M, Kristensen AB, Nielsen IK, Nielsen M, et al. Pan-specific prediction of peptide-MHC class I complex stability, a correlate of T cell immunogenicity. *J Immunol* (2016) 197(4):1517–24. doi: 10.4049/jimmunol.1600582

135. Prachar M, Justesen S, Steen-Jensen DB, Thorgrimsen S, Jurgons E, Winther O, et al. Identification and validation of 174 COVID-19 vaccine candidate epitopes reveals low performance of common epitope prediction tools. *Sci Rep* (2020) 10(1):20465. doi: 10.1038/s41598-020-77466-4

136. Luksza M, Riaz N, Makarov V, Balachandran VP, Hellmann MD, Solovyov A, et al. A neoantigen fitness model predicts tumour response to checkpoint blockade immunotherapy. *Nature* (2017) 551(7681):517–20. doi: 10.1038/nature24473

137. Luksza M, Sethna ZM, Rojas LA, Lihm J, Bravi B, Elhanati Y, et al. Neoantigen quality predicts immunoediting in survivors of pancreatic cancer. *Nature* (2022) 606(7913):389–95. doi: 10.1038/s41586-022-04735-9

138. Pande M, Wei C, Chen J, Amos CI, Lynch PM, Lu KH, et al. Cancer spectrum in DNA mismatch repair gene mutation carriers: results from a hospital based lynch syndrome registry. *Fam Cancer* (2012) 11(3):441–7. doi: 10.1007/s10689-012-9534-6

139. Sahin U, Kariko K, Tureci O. mRNA-based therapeutics–developing a new class of drugs. *Nat Rev Drug Discovery* (2014) 13(10):759–80. doi: 10.1038/nrd4278

140. Weissman D, Kariko K. mRNA: Fulfilling the promise of gene therapy. *Mol Ther* (2015) 23(9):1416–7. doi: 10.1038/mt.2015.138

141. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine. *N Engl J Med* (2020) 383(27):2603–15. doi: 10.1056/NEJMoa2034577

142. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* (2021) 384(5):403–16. doi: 10.1056/NEJMoa2035389

143. Liu MA, Zhou T, Sheets RL, Meyer H, Knezevic I. WHO informal consultation on regulatory considerations for evaluation of the quality, safety and efficacy of RNA-based prophylactic vaccines for infectious diseases, 20-22 April 2021. *Emerg Microbes Infect* (2022) 11(1):384–91. doi: 10.1080/22221751.2022.2026742

144. Geall AJ, Verma A, Otten GR, Shaw CA, Hekele A, Banerjee K, et al. Nonviral delivery of self-amplifying RNA vaccines. *Proc Natl Acad Sci U.S.A.* (2012) 109(36):14604–9. doi: 10.1073/pnas.1209367109

145. Vogel AB, Lambert L, Kinnear E, Busse D, Erbar S, Reuter KC, et al. Self-amplifying RNA vaccines give equivalent protection against influenza to mRNA vaccines but at much lower doses. *Mol Ther* (2018) 26(2):446–55. doi: 10.1016/j.ymthe.2017.11.017

146. Erasmus JH, Archer J, Fuerte-Stone J, Khandhar AP, Voigt E, Granger B, et al. Intramuscular delivery of replicon RNA encoding ZIKV-117 human monoclonal antibody protects against zika virus infection. *Mol Ther Methods Clin Dev* (2020) 18:402–14. doi: 10.1016/j.omtm.2020.06.011

147. Lundstrom K. Self-replicating RNA viruses for vaccine development against infectious diseases and cancer. *Vaccines (Basel)* (2021) 9(10):1187. doi: 10.3390/vaccines9101187

148. Palmer CD, Rappaport AR, Davis MJ, Hart MG, Scallan CD, Hong SJ, et al. Individualized, heterologous chimpanzee adenovirus and self-amplifying mRNA neoantigen vaccine for advanced metastatic solid tumors: phase 1 trial interim results. *Nat Med* (2022) 28(8):1619–29. doi: 10.1038/s41591-022-01937-6

149. Qu L, Yi Z, Shen Y, Lin L, Chen F, Xu Y, et al. Circular RNA vaccines against SARS-CoV-2 and emerging variants. *Cell* (2022) 185(10):1728–44.e16. doi: 10.1016/j.cell.2022.03.044

150. Seephetdee C, Bhukhai K, Buasri N, Leelukkanaveera P, Lerdwattanasombat P, Manopwisedjaroen S, et al. A circular mRNA vaccine prototype producing VFLIP-X spike confers a broad neutralization of SARS-CoV-2 variants by mouse sera. *Antiviral Res* (2022) 204:105370. doi: 10.1016/j.antiviral.2022.105370

151. Blakney AK, McKay PF, Yus BI, Aldon Y, Shattock RJ. Inside out: optimization of lipid nanoparticle formulations for exterior complexation and *in vivo* delivery of saRNA. *Gene Ther* (2019) 26(9):363–72. doi: 10.1038/s41434-019-0095-2

152. Alameh MG, Tombacz I, Bettini E, Lederer K, Sittplangkoon C, Wilmore JR, et al. Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. *Immunity* (2021) 54(12):2877–92.e7. doi: 10.1016/j.immuni.2021.11.001

153. Zhang H, You X, Wang X, Cui L, Wang Z, Xu F, et al. Delivery of mRNA vaccine with a lipid-like material potentiates antitumor efficacy through toll-like receptor 4 signaling. *Proc Natl Acad Sci U.S.A.* (2021) 118(6):e2005191118. doi: 10.1073/pnas.2005191118

154. Bevers S, Kooijmans SAA, Van de Velde E, Evers MJW, Seghers S, Gitz-Francois J, et al. mRNA-LNP vaccines tuned for systemic immunization induce strong antitumor immunity by engaging splenic immune cells. *Mol Ther* (2022) 30(9):3078–94. doi: 10.1016/j.ymthe.2022.07.007

155. Chen J, Ye Z, Huang C, Qiu M, Song D, Li Y, et al. Lipid nanoparticle-mediated lymph node-targeting delivery of mRNA cancer vaccine elicits robust CD8(+) T cell response. *Proc Natl Acad Sci U S A* (2022) 119(34):e2207841119. doi: 10.1073/pnas.2207841119

156. Huang X, Kong N, Zhang X, Cao Y, Langer R, Tao W. The landscape of mRNA nanomedicine. *Nat Med* (2022) 28(11):2273–87. doi: 10.1038/s41591-022-02061-1

157. Pajot A, Michel ML, Fazilleau N, Pancre V, Auriault C, Ojcius DM, et al. A mouse model of human adaptive immune functions: HLA-A2.1-/HLA-DR1-transgenic h-2 class I-/class II-knockout mice. *Eur J Immunol* (2004) 34(11):3060–9. doi: 10.1002/eji.200425463

158. Depla E, van der Aa A, Livingston BD, Crimi C, Allosery K, De Brabandere V, et al. Rational design of a multiepitope vaccine encoding T-lymphocyte epitopes for treatment of chronic hepatitis b virus infections. *J Virol* (2008) 82(1):435–50. doi: 10.1128/JVI.01505-07

159. Alexander J, Oseroff C, Sidney J, Sette A. Derivation of HLA-B*0702 transgenic mice: functional CTL repertoire and recognition of human B*0702-restricted CTL epitopes. *Hum Immunol* (2003) 64(2):211–23. doi: 10.1016/S0198-8859(02)00786-3

160. Harada N, Fukaya S, Wada H, Goto R, Osada T, Gomori A, et al. Generation of a novel HLA class I transgenic mouse model carrying a knock-in mutation at the beta2-microglobulin locus. *J Immunol* (2017) 198(1):516–27. doi: 10.4049/jimmunol.1502367

161. Lank SM, Golbach BA, Creager HM, Wiseman RW, Keskin DB, Reinherz EL, et al. Ultra-high resolution HLA genotyping and allele discovery by highly multiplexed cDNA amplicon pyrosequencing. *BMC Genomics* (2012) 13:378. doi: 10.1186/1471-2164-13-378

162. Meyskens FLJr., Curt GA, Brenner DE, Gordon G, Herberman RB, Finn O, et al. Regulatory approval of cancer risk-reducing (chemopreventive) drugs: moving what we have learned into the clinic. *Cancer Prev Res (Phila)* (2011) 4(3):311–23. doi: 10.1158/1940-6207.CAPR-09-0014

163. Cuzick J. Preventive therapy for cancer. *Lancet Oncol* (2017) 18(8):e472–e82. doi: 10.1016/S1470-2045(17)30536-3

164. Bruni D, Angell HK, Galon J. The immune contexture and immunoscore in cancer prognosis and therapeutic efficacy. *Nat Rev Cancer* (2020) 20(11):662–80. doi: 10.1038/s41568-020-0285-7

165. Hiam-Galvez KJ, Allen BM, Spitzer MH. Systemic immunity in cancer. *Nat Rev Cancer* (2021) 21(6):345–59. doi: 10.1038/s41568-021-00347-z

166. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* (2006) 313(5795):1960–4. doi: 10.1126/science.1129139

167. Bindea G, Mlecnik B, Fridman WH, Pages F, Galon J. Natural immunity to cancer in humans. *Curr Opin Immunol* (2010) 22(2):215–22. doi: 10.1016/j.coi.2010.02.006

168. Galon J, Angell HK, Bedognetti D, Marincola FM. The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. *Immunity* (2013) 39(1):11–26. doi: 10.1016/j.immuni.2013.07.008

169. Chao JL, Savage PA. Unlocking the complexities of tumor-associated regulatory T cells. *J Immunol* (2018) 200(2):415–21. doi: 10.4049/jimmunol.1701188

170. Ostrand-Rosenberg S, Fenselau C. Myeloid-derived suppressor cells: Immune-suppressive cells that impair antitumor immunity and are sculpted by their environment. *J Immunol* (2018) 200(2):422–31. doi: 10.4049/jimmunol.1701019

171. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. *Nat Rev Immunol* (2021) 21(8):485–98. doi: 10.1038/s41577-020-00490-y

172. Barth SD, Schulze JJ, Kuhn T, Raschke E, Husing A, Johnson T, et al. Treg-mediated immune tolerance and the risk of solid cancers: Findings from EPIC-Heidelberg. *J Natl Cancer Inst* (2015) 107(11):djv224. doi: 10.1093/jnci/djv224

173. Cader FZ, Hu X, Goh WL, Wienand K, Ouyang J, Mandato E, et al. A peripheral immune signature of responsiveness to PD-1 blockade in patients with classical Hodgkin lymphoma. *Nat Med* (2020) 26(9):1468–79. doi: 10.1038/s41591-020-1006-1

174. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* (2014) 515(7528):568–71. doi: 10.1038/nature13954

175. Watson RA, Tong O, Cooper R, Taylor CA, Sharma PK, de Los Aires AV, et al. Immune checkpoint blockade sensitivity and progression-free survival associates with baseline CD8(+) T cell clone size and cytotoxicity. *Sci Immunol* (2021) 6(64):eabj8825. doi: 10.1126/sciimmunol.abj8825

176. Meyer C, Cagnon L, Costa-Nunes CM, Baumgaertner P, Montandon N, Leyvraz L, et al. Frequencies of circulating MDSC correlate with clinical outcome of melanoma patients treated with ipilimumab. *Cancer Immunol Immunother* (2014) 63(3):247–57. doi: 10.1007/s00262-013-1508-5

177. Gebhardt C, Sevko A, Jiang H, Lichtenberger R, Reith M, Tarnanidis K, et al. Myeloid cells and related chronic inflammatory factors as novel predictive markers in melanoma treatment with ipilimumab. *Clin Cancer Res* (2015) 21(24):5453–9. doi: 10.1158/1078-0432.CCR-15-0676

178. Martens A, Wistuba-Hamprecht K, Geukes Foppen M, Yuan J, Postow MA, Wong P, et al. Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. *Clin Cancer Res* (2016) 22(12):2908–18. doi: 10.1158/1078-0432.CCR-15-2412

179. Hogan SA, Courtier A, Cheng PF, Jaberg-Bentele NF, Goldinger SM, Manuel M, et al. Peripheral blood TCR repertoire profiling may facilitate patient stratification for immunotherapy against melanoma. *Cancer Immunol Res* (2019) 7(1):77–85. doi: 10.1158/2326-6066.CIR-18-0136

180. Spitzer MH, Carmi Y, Reticker-Flynn NE, Kwek SS, Madhireddy D, Martins MM, et al. Systemic immunity is required for effective cancer immunotherapy. *Cell* (2017) 168(3):487–502.e15. doi: 10.1016/j.cell.2016.12.022

181. Huang AC, Postow MA, Orlowski RJ, Mick R, Bengsch B, Manne S, et al. T-Cell invigoration to tumour burden ratio associated with anti-PD-1 response. *Nature* (2017) 545(7652):60–5. doi: 10.1038/nature22079

182. Fairfax BP, Taylor CA, Watson RA, Nassiri I, Danielli S, Fang H, et al. Peripheral CD8(+) T cell characteristics associated with durable responses to immune checkpoint blockade in patients with metastatic melanoma. *Nat Med* (2020) 26(2):193–9. doi: 10.1038/s41591-019-0734-6

183. Valpione S, Galvani E, Tweedy J, Mundra PA, Banyard A, Middlehurst P, et al. Immune-awakening revealed by peripheral T cell dynamics after one cycle of immunotherapy. *Nat Cancer* (2020) 1(2):210–21. doi: 10.1038/s43018-019-0022-x

184. Gebhardt T, Wakim LM, Eidsmo L, Reading PC, Heath WR, Carbone FR. Memory T cells in nonlymphoid tissue that provide enhanced local immunity during infection with herpes simplex virus. *Nat Immunol* (2009) 10(5):524–30. doi: 10.1038/ni.1718

185. Jiang X, Clark RA, Liu L, Wagers AJ, Fuhlbrigge RC, Kupper TS. Skin infection generates non-migratory memory CD8+ T(RM) cells providing global skin immunity. *Nature* (2012) 483(7388):227–31. doi: 10.1038/nature10851

186. Ariotti S, Hogenbirk MA, Dijkgraaf FE, Visser LL, Hoekstra ME, Song JY, et al. T Cell memory. skin-resident memory CD8(+) T cells trigger a state of tissue-wide pathogen alert. *Science* (2014) 346(6205):101–5. doi: 10.1126/science.1254803

187. Yenyuwadee S, Sanchez-Trincado Lopez JL, Shah R, Rosato PC, Boussiotis VA. The evolving role of tissue-resident memory T cells in infections and cancer. *Sci Adv* (2022) 8(33):eabo5871. doi: 10.1126/sciadv.abo5871

188. Mami-Chouaib F, Blanc C, Corgnac S, Hans S, Malenica I, Granier C, et al. Resident memory T cells, critical components in tumor immunology. *J Immunother Cancer* (2018) 6(1):87. doi: 10.1186/s40425-018-0399-6

189. Park SL, Gebhardt T, Mackay LK. Tissue-resident memory T cells in cancer immunosurveillance. *Trends Immunol* (2019) 40(8):735–47. doi: 10.1016/j.it.2019.06.002

190. Kok L, Masopust D, Schumacher TN. The precursors of CD8(+) tissue resident memory T cells: from lymphoid organs to infected tissues. *Nat Rev Immunol* (2022) 22(5):283–93. doi: 10.1038/s41577-021-00590-3

191. Djenidi F, Adam J, Goubar A, Durgeau A, Meurice G, de Montpreville V, et al. CD8+CD103+ tumor-infiltrating lymphocytes are tumor-specific tissue-resident memory T cells and a prognostic factor for survival in lung cancer patients. *J Immunol* (2015) 194(7):3475–86. doi: 10.4049/jimmunol.1402711

192. Nizard M, Roussel H, Diniz MO, Karaki S, Tran T, Voron T, et al. Induction of resident memory T cells enhances the efficacy of cancer vaccine. *Nat Commun* (2017) 8:15221. doi: 10.1038/ncomms15221

193. Menares E, Galvez-Cancino F, Caceres-Morgado P, Ghorani E, Lopez E, Diaz X, et al. Tissue-resident memory CD8(+) T cells amplify anti-tumor immunity by triggering antigen spreading through dendritic cells. *Nat Commun* (2019) 10(1):4401. doi: 10.1038/s41467-019-12319-x

194. Toh JWT, Ferguson AL, Spring KJ, Mahajan H, Palendira U. Cytotoxic CD8+ T cells and tissue resident memory cells in colorectal cancer based on microsatellite instability and BRAF status. *World J Clin Oncol* (2021) 12(4):238–48. doi: 10.5306/wjco.v12.i4.238

195. Boddupalli CS, Bar N, Kadaveru K, Krauthammer M, Pornputtapong N, Mai Z, et al. Interlesional diversity of T cell receptors in melanoma with immune checkpoints enriched in tissue-resident memory T cells. *JCI Insight* (2016) 1(21):e88955. doi: 10.1172/jci.insight.88955

196. Caushi JX, Zhang J, Ji Z, Vaghasia A, Zhang B, Hsiue EH, et al. Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers. *Nature* (2021) 596(7870):126–32. doi: 10.1038/s41586-021-03752-4

197. Sun YY, Peng S, Han L, Qiu J, Song L, Tsai Y, et al. Local HPV recombinant vaccinia boost following priming with an HPV DNA vaccine enhances local HPV-specific CD8+ T-cell-Mediated tumor control in the genital tract. *Clin Cancer Res* (2016) 22(3):657–69. doi: 10.1158/1078-0432.CCR-15-0234

198. Galvez-Cancino F, Lopez E, Menares E, Diaz X, Flores C, Caceres P, et al. Vaccination-induced skin-resident memory CD8(+) T cells mediate strong protection against cutaneous melanoma. *Oncoimmunology* (2018) 7(7):e1442163. doi: 10.1080/2162402X.2018.1442163

199. Kinker GS, Vitiello GAF, Ferreira WAS, Chaves AS, Cordeiro de Lima VC, Medina TDS. B cell orchestration of anti-tumor immune responses: A matter of cell localization and communication. *Front Cell Dev Biol* (2021) 9:678127. doi: 10.3389/fcell.2021.678127

200. Ma P, Beatty PL, McKolanis J, Brand R, Schoen RE, Finn OJ. Circulating myeloid derived suppressor cells (MDSC) that accumulate in premalignancy share phenotypic and functional characteristics with MDSC in cancer. *Front Immunol* (2019) 10:1401. doi: 10.3389/fimmu.2019.01401

201. Mascaux C, Angelova M, Vasaturo A, Beane J, Hijazi K, Anthoine G, et al. Immune evasion before tumour invasion in early lung squamous carcinogenesis. *Nature* (2019) 571(7766):570–5. doi: 10.1038/s41586-019-1330-0

202. Pang X, Fan HY, Tang YL, Wang SS, Cao MX, Wang HF, et al. Myeloid derived suppressor cells contribute to the malignant progression of oral squamous cell carcinoma. *PLoS One* (2020) 15(2):e0229089. doi: 10.1371/journal.pone.0229089

203. Chang K, Taggart MW, Reyes-Uribe L, Borras E, Riquelme E, Barnett RM, et al. Immune profiling of premalignant lesions in patients with lynch syndrome. *JAMA Oncol* (2018) 4(8):1085–92. doi: 10.1001/jamaoncol.2018.1482

204. Staffa L, Echterdiek F, Nelius N, Benner A, Werft W, Lahrmann B, et al. Mismatch repair-deficient crypt foci in lynch syndrome–molecular alterations and association with clinical parameters. *PLoS One* (2015) 10(3):e0121980. doi: 10.1371/journal.pone.0121980

205. Ahadova A, Gallon R, Gebert J, Ballhausen A, Endris V, Kirchner M, et al. Three molecular pathways model colorectal carcinogenesis in lynch syndrome. *Int J Cancer* (2018) 143(1):139–50. doi: 10.1002/ijc.31300

206. Ahadova A, Seppala TT, Engel C, Gallon R, Burn J, Holinski-Feder E, et al. The "unnatural" history of colorectal cancer in lynch syndrome: Lessons from colonoscopy surveillance. *Int J Cancer* (2021) 148(4):800–11. doi: 10.1002/ijc.33224

207. Cecil DL, Gad EA, Corulli LR, Drovetto N, Lubet RA, Disis ML. COX-2 inhibitors decrease expression of PD-L1 in colon tumors and increase the influx of type I tumor-infiltrating lymphocytes. *Cancer Prev Res (Phila)* (2022) 15(4):225–31. doi: 10.1158/1940-6207.CAPR-21-0227

208. Roudko V, Cimen Bozkus C, Greenbaum B, Lucas A, Samstein R, Bhardwaj N. Lynch syndrome and MSI-h cancers: From mechanisms to "Off-The-Shelf" cancer vaccines. *Front Immunol* (2021) 12:757804. doi: 10.3389/fimmu.2021.757804

209. Guerriero JL, Sotayo A, Ponichtera HE, Castrillon JA, Pourzia AL, Schad S, et al. Class IIa HDAC inhibition reduces breast tumours and metastases through anti-tumour macrophages. *Nature* (2017) 543(7645):428–32. doi: 10.1038/nature21409

210. Chen S, Song Z, Zhang A. Small-molecule immuno-oncology therapy: Advances, challenges and new directions. *Curr Top Med Chem* (2019) 19(3):180–5. doi: 10.2174/1568026619666190308131805

211. Verma V, Jafarzadeh N, Boi S, Kundu S, Jiang Z, Fan Y, et al. MEK inhibition reprograms CD8(+) T lymphocytes into memory stem cells with potent antitumor effects. *Nat Immunol* (2021) 22(1):53–66. doi: 10.1038/s41590-020-00818-9

212. Sasikumar PG, Ramachandra M. Small molecule agents targeting PD-1 checkpoint pathway for cancer immunotherapy: Mechanisms of action and other considerations for their advanced development. *Front Immunol* (2022) 13:752065. doi: 10.3389/fimmu.2022.752065

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 06 April 2023
DOI 10.3389/fonc.2023.1147657

Check for updates

OPEN ACCESS

EDITED BY
Christina Therkildsen,
Copenhagen University Hospital, Denmark

REVIEWED BY
Jon Ambæk Durhuus,
Hvidovre Hospital, Denmark
Stephanie M. McGregor,
University of Wisconsin-Madison,
United States

*CORRESPONDENCE
Courtney J. Riedinger
courtney.riedinger@osumc.com

SPECIALTY SECTION
This article was submitted to
Cancer Genetics,
a section of the journal
Frontiers in Oncology

RECEIVED 19 January 2023
ACCEPTED 10 March 2023
PUBLISHED 06 April 2023

CITATION
Riedinger CJ, Brown M, Haight PJ,
Backes FJ, Cohn DE, Goodfellow PJ and
Cosgrove CM (2023) Epigenetic MMR
defect identifies a risk group not
accounted for through traditional
risk stratification algorithms in
endometrial cancer.
Front. Oncol. 13:1147657.
doi: 10.3389/fonc.2023.1147657

COPYRIGHT
© 2023 Riedinger, Brown, Haight, Backes,
Cohn, Goodfellow and Cosgrove. This is an
open-access article distributed under the
terms of the Creative Commons Attribution
License (CC BY). The use, distribution or
reproduction in other forums is permitted,
provided the original author(s) and the
copyright owner(s) are credited and that
the original publication in this journal is
cited, in accordance with accepted
academic practice. No use, distribution or
reproduction is permitted which does not
comply with these terms.

Epigenetic MMR defect identifies a risk group not accounted for through traditional risk stratification algorithms in endometrial cancer

Courtney J. Riedinger[1]*, Morgan Brown[2], Paulina J. Haight[1], Floor J. Backes[1], David E. Cohn[1], Paul J. Goodfellow[1] and Casey M. Cosgrove[1]

[1]Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, Arthur G. James Cancer Hospital, The Ohio State University Wexner Medical Center, Columbus, OH, United States, [2]Department of Obstetrics and Gynecology, The Ohio State University Wexner Medical Center, Columbus, OH, United States

Purpose: We sought to evaluate the contribution of mismatch repair (MMR) status to traditional risk stratification algorithms used to predict nodal involvement and recurrence in a large single-institution cohort.

Methods: Endometrioid endometrial cancer (EC) cases from 2014-2020 were evaluated. MMR immunohistochemistry (IHC) was performed universally. Uterine factors assessed in the Mayo criteria were used to retrospectively classify patients as low or high risk for lymphatic spread. Patients were classified according to risk for recurrence using GOG 99 and PORTEC criteria. Associations were evaluated using chi-square and t-tests and contributing factors assessed using logistic regression models.

Results: 1,514 endometrioid EC were evaluated; 392 (25.9%) were MMR (MMR) deficient of which 80.4% of MMR defects were associated with epigenetic silencing of *MLH1*. Epigenetic MMR defects were significantly more likely to be high risk for lymph node (LN) metastasis based on Mayo criteria (74.9% vs 60.6%, p=<0.001) and with the presence of LN metastasis (20.3 vs 10.5%, p=0.003) compared to MMR proficient tumors. Tumors with epigenetic MMR defects were significantly more likely to be classified as high or high intermediate risk using GOG99 and PORTEC criteria. Furthermore, cases with epigenetic MMR defects classified as low or low intermediate risk were significantly more likely to recur (GOG99 p=0.013; PORTEC p=0.008) and independently associated with worse disease-free survival (DFS). MMR status was found to be independently associated with worse DFS (HR 1.90; 95% CI 1.34-2.70; p=0.003) but not overall survival.

Conclusion: While MMR deficient EC has been associated with poor prognostic features in prior reports; we demonstrate that only epigenetic MMR defects have poorer outcomes. Epigenetic MMR defect were independently associated with lymph node metastasis after controlling for risk criteria. Epigenetic MMR

deficiency was found to be an independent predictor of recurrence beyond the factors considered in traditional risk stratification algorithms. Traditional uterine-based risk stratification algorithms may not fully reflect the risk for recurrence in MMR deficient tumors. Consideration should be given to implementing MMR status and *MLH1* hypermethylation alongside traditional risk stratification algorithms. Performing MMR IHC on preoperative pathologic specimens may aid in risk stratification and patient counseling.

KEYWORDS

mismatch repair deficiency (MMR), epigenetic loss, Lynch syndrome, biomarker, risk stratification, endometrial cancer

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in the U.S. and more than 66,000 new cases will be diagnosed in 2023. MMR (MMR) deficiency is common in EC, occurring in 20-40% of cases (1, 2). Determination of MMR status in EC has several clinical implications. Loss of expression of MMR proteins may be associated with inherited germline defects in MMR genes (*MLH1, MSH2, MSH6, PMS2*). Approximately 3-5% of EC may be attributed to Lynch Syndrome (LS), a hereditary cancer predisposition caused by mutations in mismatch repair (MMR) genes. Women with LS have up to a 60% lifetime risk for developing EC as well as a significant risk for colorectal, ovarian, stomach, and other cancers (3–6). EC serves as an important 'sentinel' cancer and is the first cancer diagnosed in approximately 50% of women with LS (6, 7). While MMR deficiency in EC is common, the majority of cases can be explained by epigenetic silencing of the *MLH1* promoter rather than germline defects (1, 2, 8, 9). While $BRAF^{V600E}$ mutations are frequently implicated in sporadic colorectal cancer (10), *BRAF* mutations are very rare in EC (0.1%) and testing is not recommended as part of universal screening for LS (11, 12).

Outside of genetic screening, MMR status is an important prognostic biomarker (13–15) and can be used to predict response to immunotherapy (16, 17). Currently, MMR testing is recommended by the National Comprehensive Cancer Network (NCCN) as a complement to morphologic assessment of EC and is used to separate EC into one of four molecular subgroups (*POLE* mutated, MMR-deficient/Microsatellite instability-high, copy number low, and copy number high) (18, 19). Our group, and others, have reported on the association between MMR deficiency and a number of poor prognostic indicators routinely used to guide the decision for adjuvant therapy in endometrioid EC. Epigenetic MMR defects have been associated with diagnosis at an older age, the presence of lymphovascular space invasion (LVSI), and higher-grade tumors, as well as diagnosis at a more advanced stage. EC with *MLH1* hypermethylation has also been associated with larger tumor volumes increasing the risk for lymph node metastasis (9, 20). However, even with these poor prognostic features, data regarding outcomes in EC with MMR deficiency and epigenetic MMR defects have been inconsistent (9, 21). While many groups have reported on reduced recurrence free survival in EC with MMR defects others have reported that there is no effect or even an improvement in OS in these tumors (11, 22–33).

We sought to determine if MMR status might add to traditional risk stratification algorithms used to predict risk for lymph node metastasis and recurrence in a large, single-institution cohort.

Materials and methods

This was an institutional review board-approved retrospective review from the Ohio State University Comprehensive Cancer Center (OSUCCC) from June 1, 2014 to December 31, 2020. All patients who underwent surgery for an EC diagnosis at our institution were included. Clinical and demographic data were abstracted from medical records. Electronic health information exchange (HIE) was used to access medical records from outside institutions where available. A portion of this cohort was included in previous reports (20, 22).

Universal MMR IHC testing for protein expression of MLH1, PMS2, MSH2, and MSH6 was performed clinically on all EC specimens for LS screening as standard of care. Tumors with loss of expression of MLH1 or PMS2 on IHC underwent reflex *MLH1* methylation testing using methylation-specific PCR to triage for genetics referral. MMR status of tumors was classified as MMR proficient (normal) if there was intact expression of MMR proteins. Patients' tumors with loss of MLH1/PMS2 on IHC and methylation of the *MLH1* promoter region were classified as having an epigenetic MMR defect. Tumors with abnormal IHC without *MLH1* methylation were classified as MMR deficient due to a probable MMR mutation (probable Lynch syndrome or double somatic mutation).

The criteria established by Mariani et al. from the Mayo Clinic (i.e. tumor diameter, grade, and depth of invasion) were used to retrospectively classify patients as low or high risk for lymphatic spread (23). Patients were classified as low risk for lymph node metastasis if they were without evidence of extrauterine disease, with primary tumor diameter ≤2cm, FIGO grade 1 or 2 histology, and ≤50% myometrial invasion. Tumor grade and depth of

myometrial invasion were abstracted from the final pathology report. Tumor size was based on hysterectomy gross tumor specimen measurements recorded by the evaluating pathologist. Tumor volume was calculated using the maximum tumor measurements for 3 lengths as previously described (20). Subjects were classified according to GOG99 and PORTEC risk criteria as previously reported (34–36). Briefly, patients were classified as high intermediate risk (HIR) by GOG 99 depending on age and the number of risk factors (grade 2 or 3 tumor, the presence of LVSI, and outer 1/3 myometrial invasion). Patients were classified as high intermediate risk by PORTEC if they had 2 of 3 clinicopathologic factors: age > 60 years, ≥50% myometrial invasion, and grade 3 histology.

Clinical-pathologic relationships were assessed using $\chi 2$, Fisher's exact test, and t test. Where data were not normally distributed, the Wilcoxon signed-rank test was utilized. The Kaplan-Meier product limit was used to estimate survival. The log-rank test was used to test for differences in survival. Multivariable logistic regression models were developed, and odds ratios (ORs) were used to evaluate the risk factors associated with recurrence. Cox proportional hazard models were used to assess variables associated with disease-free (DFS) and overall survival (OS). All statistical analyses were performed using JMP® Pro, Version 15.2.0. SAS Institute Inc., Cary, NC, 2019.

Results

Data was collected for 1,718 ECs; for the purposes of this study analyses were limited to endometrioid histology EC (N=1,514). The median follow-up time was 2.5 years (range 20 days to 7.8 years). The clinical and pathologic features of the entire cohort, stratified by MMR status are presented in Table 1. Most patients were obese (82%), were stage I at diagnosis (83%), and had grade I tumors (81%). Three-hundred ninety-two (25.9%) patients' tumors demonstrated MMR defect based on IHC. Eighty percent (315/392) of those were associated with *MLH1* hypermethylation and classified as epigenetic MMR defects. Seventy-seven patients had MMR IHC loss of expression without *MLH1* hypermethylation suggestive of MMR mutations. IHC staining for these cases revealed 15 with loss of MLH1/PMS2 without MLH1 hypermethylation, 9 with isolated loss of PMS2 without MLH1 hypermethylation, 25 with loss of MSH2/MSH6, and 28 with isolated loss of MSH6 staining. Of those 77 patients, germline testing results were available in 53 cases, 38 of whom (2.5% of the entire cohort) had confirmed LS. *MSH6*-related LS was diagnosed in 15 cases, *PMS2*-related LS in 14 cases, *MSH2*-related LS in 7 cases, and *MLH1*-related LS in 2 cases.

The only significant difference between patients with a probable MMR deficiency and those who were MMR proficient was age (median 56 vs 60 years, p=0.009) and BMI (median 32.2 vs 38.3, p=0.003). EC with probable MMR deficiency did not differ from MMR proficient EC in terms of stage, grade, LVSI, the receipt of adjuvant therapy, Mayo risk criteria, GOG 99, or PORTEC risk criteria. Comparatively, ECs with epigenetic loss of *MLH1* were significantly more likely to be diagnosed at a more advanced stage (23.8%), with higher grade tumors (12.4%), with LVSI (37.5%), and to receive adjuvant therapy (41.2%) (Table 1).

MMR status and traditional risk stratification algorithms

Mayo criteria

Given the reported increased risk of lymph node (LN) metastasis in EC with epigenetic loss of *MLH1* we evaluated the contribution of MMR status to Mayo criteria. Mayo criteria published by Mariani et al. has been used to identify patients which may safely be excluded from routine lymphadenectomy due to low risk of LN metastasis (37). We evaluated 1,477 endometrioid EC without preoperative evidence of advanced disease (without evidence of metastatic disease or lymphadenopathy on preoperative imaging). The majority of EC in our cohort (77.8%) underwent LN assessment (sentinel lymph node biopsy or full lymphadenectomy) regardless of MAYO criteria risk. MMR deficient EC were significantly more likely to be deemed high-risk for lymph node metastasis by Mayo criteria (74.9% vs 60.6%, p=≤0.001) (Table 1). However, there was no significant difference in the characteristics that resulted in exclusion from the low-risk group (ie. Tumor size, myometrial invasion, grade) between MMR deficient and MMR proficient EC. In addition, in patients at high risk for lymphatic spread by Mayo criteria, ECs with epigenetic MMR defect were significantly more likely to have LN metastasis (20.3% vs 10.5%, p=0.003). There was no significant difference in the rate of LN metastasis between patients with probable MMR mutation and MMR proficient EC after selecting for those at high-risk by Mayo criteria (15.4% vs 10.5%, p=0.369). While Mayo criteria is not used routinely to omit lymph node assessment in our practice due to high utilization of sentinel lymphadenectomy, there was a significantly higher rate of lymphadenectomy in patients with MMR deficient EC compared to MMR proficient EC (83.7% vs 74.1%, p=≤0.001) reflecting the impact that intraoperative assessment of tumor volume may have in surgical decision making. Sixty-seven percent of patients at low risk by Mayo criteria underwent surgical lymph node assessment. There were 4 cases of lymph node metastases in patients deemed low risk by Mayo criteria; two of these occurred in patients with epigenetic MMR defect, and two in MMR proficient EC. The false negative rate of Mayo criteria in epigenetic MMR defects was 3.9% (compared to 0.7% in MMR proficient) (HR 5.44, 95% CI 0.78-37.8, p=0.105). There were two retroperitoneal recurrences that could be related to undiagnosed lymphatic spread in patients who did not undergo lymph node assessment; both patients were high-risk by Mayo criteria but did not undergo lymphatic dissection due to inadequate visualization and medical comorbidities. Both patients had MMR deficient tumors (one epigenetic loss and one with a probable MMR mutation). A nominal logistic regression model was used to evaluate the risk for lymph node metastasis. Epigenetic MMR defect was found to be an independent risk factor for lymph node metastasis (HR 2.52; 95% CI 1.65-3.85; p=≤0.001) after controlling for risk group by Mayo criteria, LVSI, and tumor volume (Table 2).

GOG99 and PORTEC risk classification

Adjuvant radiation has not been shown to improve survival in early-stage disease (34–36) and the role of adjuvant therapy in

10.3389/fonc.2023.1147657

TABLE 1 Clinical-pathologic features of Endometrioid EC by MMR status.

Clinical-pathologic features	MMR proficient	Epigenetic loss *MLH1*	Probable MMR mutation	*p* value
	N = 1122 (%)	*N* = 315 (%)	*N* = 77 (%)	
Age				
Mean (SD)	59.6 (11.16)	64.67 (9.55)	55.08 (9.23)	≤0.001
Median (Range)	60.0 (25-94)	64.0 (35-90)	56.0 (37-76)	
BMI				
Mean (SD)	39.08 (10.61)	37.3 (8.65)	34.2 (9.69)	≤0.001
Median (Range)	38.28 (18.6-81.3)	36.4 (19.4-66.4)	32.2 (20.4-62.4)	
Racial/Ethnic Group				0.240
White	1060 (94.5)	296 (93.8)	71 (92.2)	
Black	34 (3.0)	14 (4.4)	2 (2.6)	
Asian	12 (1.1)	4 (1.3)	4 (5.2)	
Other	16 (1.4)	1 (0.3)	0	
Stage				≤0.001
I	988 (88.1)	229 (72.7)	66 (85.7)	
II	31 (2.8)	11 (3.5)	2 (2.6)	
III	86 (7.7)	57 (18.1)	7 (9.1)	
IV	17 (1.5)	18 (5.7)	2 (2.6)	
FIGO Grade				≤0.001
1	962 (85.7)	199 (63.2)	65 (84.4)	
2	116 (10.3)	77 (24.4)	6 (7.8)	
3	44 (3.9)	39 (12.4)	6 (7.8)	
LVSI				
Present	164 (14.6)	118 (37.5)	12 (15.6)	≤0.001
Absent	958 (85.4)	197 (62.5)	65 (84.4)	
Mayo criteria				
High risk	690 (61.5)	249 (79.0)	48 (62.3)	≤0.001
Low risk	432 (38.5)	66 (21.0)	29 (37.7)	
GOG 99 risk classification				
Low risk	292 (26.0)	40 (12.7)	19 (24.7)	≤0.001
Low intermediate risk	621 (55.4)	133 (42.2)	46 (59.7)	
High intermediate risk	104 (9.3)	67 (21.3)	3 (3.9)	
High risk	105 (9.4)	75 (23.8)	9 (11.7)	
PORTEC risk classification				
Low risk	832 (74.3)	175 (55.6)	58 (75.3)	≤0.001
High intermediate risk	146 (13.0)	43 (13.7)	7 (9.1)	
High risk	142 (12.7)	97 (30.8)	12 (15.6)	
Adjuvant therapy				
Chemotherapy	60 (5.4)	29 (9.2)	7 (9.1)	≤0.001

(Continued)

TABLE 1 Continued

Clinical-pathologic features	MMR proficient *N* = 1122 (%)	Epigenetic loss *MLH1* *N* = 315 (%)	Probable MMR mutation *N* = 77 (%)	*p* value
Chemo + radiation	69 (6.2)	49 (15.6)	6 (7.8)	
Radiation	105 (9.4)	50 (15.9)	9 (11.7)	
Other	6 (0.5)	2 (0.6)	0	
None	882 (78.6)	185 (58.7)	55 (71.4)	
Recurrence/Progression				
Yes	43 (3.8)	48 (15.2)	3 (3.9)	≤0.001
No	1079 (96.2)	267 (84.8)	74 (96.1)	

early-stage endometrial cancer remains uncertain. The GOG99 and PORTEC studies evaluated the role of adjuvant therapy in early-stage endometrial cancer (34–36) and identified patients that would benefit from adjuvant radiation to decrease the risk for pelvic recurrence. These trials arrived at different (but overlapping) criteria to determine high intermediate risk. We categorized the 1,327 early-stage endometrioid EC in our cohort according to the GOG99 and PORTEC criteria: Eighty seven percent were deemed low or low intermediate risk by GOG99 criteria, and 80.9% low risk by PORTEC criteria. MMR deficient ECs were significantly more likely to meet GOG99 HIR criteria (23.1% vs 10.3%, p=≤0.001) and high risk or HIR by PORTEC (23.4% vs 17.8%, p=0.004) (Table 1). A nominal logistic regression model was used to evaluate the risk of recurrence after controlling for risk classification. After controlling for GOG99 classification and PORTEC classification, MMR status as a dichotomous variable was found to be an independent risk factor for recurrence, (HR 2.34; 95% CI 1.25-4.39; p=0.008). When MMR status was evaluated as a trichotomous variable only epigenetic loss (rather than probable MMR mutation) remained independently associated with recurrence (HR 2.74; 95% CI 1.44-5.25; p=0.002) after controlling for GOG99 and PORTEC classification (Table 2). Epigenetic loss of *MLH1* also demonstrated significant association with recurrence after correcting for receipt of any adjuvant therapy and type of adjuvant therapy. Indeed, EC with epigenetic MMR defect had a statistically and clinically meaningful increased rate of recurrence in early-stage EC compared to MMR proficient EC (7.9% vs 2.4%, p=0.005) (Table 3).

TABLE 2 Multivariate analysis risk for LN metastasis and risk for recurrence in endometrioid EC.

Variable	Risk for Lymph Node Metastasis			Risk for Recurrence		
	Hazard ratio	95% CI	*p* value	Hazard ratio	95% CI	*p* value
Mayo criteria (high risk vs low risk)	5.60	4.62-24.78	≤0.001*			
Tumor volume (continuous variable)	1.40	1.21-1.62	0.003*	0.99	0.99-1.01	0.153
LVSI (present vs absent)	6.30	2.81-18.67	≤0.001*	2.37	1.34-4.19	0.003*
MMR status (MMR deficient vs MMR proficient)	1.99	1.31-3.03	0.001*	2.34	1.25-4.39	0.008*
MMR status (epigenetic vs MMR proficient)	2.52	1.65-3.85	≤0.001*	2.74	1.44-5.25	0.002*
MMR status (probable MMR mutation vs MMR normal)	1.29	0.49-3.44	0.600	0.66	0.09-4.95	0.683
Age (continuous variable)	0.50	0.12-2.02	0.333	1.03	0.94-1.35	0.238
BMI (continuous variable)	0.99	0.97-1.02	0.532	0.99	0.96-1.03	0.747
FIGO Grade (grade 1 & 2 vs 3)	0.38	0.21-0.68	0.001*	0.4	0.22-0.73	0.0027*
Stage (III/IV vs I/II)				3.68	2.08-6.54	≤0.001*
Adjuvant therapy (therapy vs no therapy)				0.67	0.19-0.37	0.193
GOG99 (HIR vs LIR or Low risk)[a]				3.94	2.09-7.44	≤0.001*
PORTEC (High risk or HIR vs Low risk)[a]				3.62	1.98-6.61	≤0.001*

*Denotes statistical significance. Data from N=1,514 endometrioid endometrial cancers except for [a] which evaluates 1,327 stage I and II endometrial cancers. N=1,178 EC underwent lymph node dissection, N=112 EC with lymph node metastasis. N=97 with recurrent disease.
LN, (Lymph Node); LVSI, (Lymphovascular space invasion); MMR, (Mismatch Repair); HIR, (High intermediate risk); LIR, (Low intermediate risk).

TABLE 3 Recurrence rates early stage endometrioid histology EC by MMR status.

Clinical-pathologic features	MMR proficient *N* = 1019 (%)	Epigenetic loss *MLH1* *N* = 240 (%)	Probable MMR mutation *N* = 68 (%)	*p* value
GOG 99 risk classification				
Low or Low intermediate risk				
Recurrence	15 (1.6)	10 (5.8)	1 (1.5)	0.013
No recurrence	896 (98.4)	163 (94.2)	64 (98.5)	
High intermediate risk				
Recurrence	9 (8.3)	9 (13.4)	0	0.410
No recurrence	99 (91.7)	58 (86.6)	3 (100.0)	
PORTEC risk classification				
Low risk				
Recurrence	12 (1.4)	10 (5.7)	1 (1.7)	0.010
No recurrence	822 (98.6)	165 (94.3)	57 (98.3)	
High or High intermediate risk				
Recurrence	12 (6.5)	9 (13.8)	0	0.090
No recurrence	173 (93.5)	56 (86.2)	10 (100.0)	

Survival

MMR deficient endometrioid EC had worse DFS (Figure 1) and OS than MMR proficient EC (OS data not shown). Only 65.0% of MMR deficient tumors were disease free at 5 years compared to 88.1% of MMR proficient tumors (p=≤0.001). This detrimental effect appears to be driven by the behavior of EC with epigenetic loss of *MLH1*. The 5-year DFS of EC with epigenetic loss was 57.5% compared to 85% in EC with probable MMR mutation and 88.1% in MMR proficient EC (p=≤0.001). The 5-year OS for EC with epigenetic loss of MLH1 was 74.6% compared to 89.1% for EC with probable MMR mutation and 90% for MMR proficient EC (p=0.003). Univariate analysis revealed that MMR status, age, BMI, stage, grade, and LVSI were significantly associated with survival. When the six factors significant in univariate analysis were included in multivariable analysis (along with adjuvant therapy) MMR status was found to be independently associated with DFS but not OS (HR 1.90; 95% CI 1.34-2.70; p=0.003) (Table 4).

In early-stage endometrioid EC, MMR deficiency was associated with significantly worse OS and DFS (Supplementary Figure S1). The effect of MMR deficiency on DFS was evaluated *via* Cox proportional hazard ratios; epigenetic loss of *MLH1* was independently associated with worse DFS in early-stage EC after controlling for GOG99 and PORTEC risk classification (HR 2.75; 95% CI 1.69-4.48; p=≤0.001). Most striking, in patients at low and low-intermediate risk by GOG 99 criteria there was a significantly increased risk for recurrence and worse DFS (5-year DFS 80.8% vs 94.6% at 5 years, p=0.004). The effect of MMR deficiency on

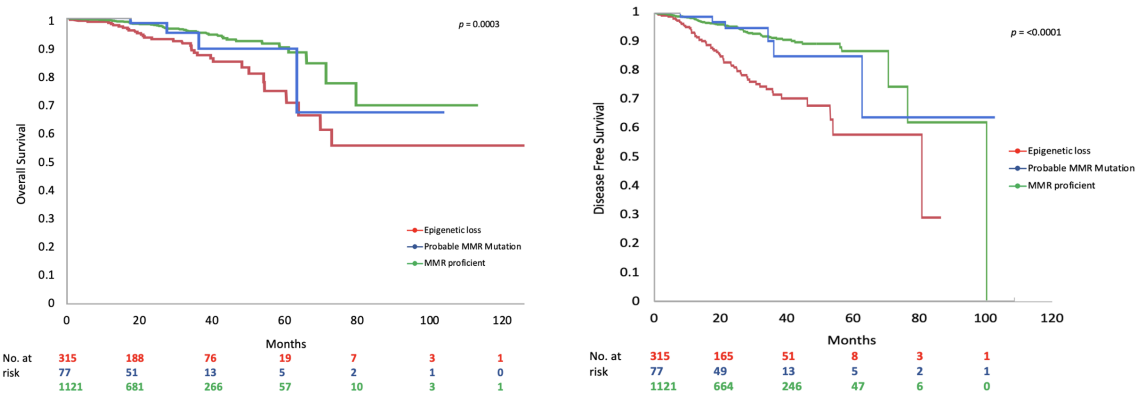


FIGURE 1
Overall survival and disease-free survival by MMR class.

TABLE 4 Multivariate analysis for endometrioid EC disease-free survival and overall survival.

Variable	Disease-free survival			Overall Survival		
	Hazard ratio	95% CI	*p* value	Hazard ratio	95% CI	*p* value
Age (continuous variable)	1.04	1.02-1.06	≤0.001*	1.05	1.02-1.11	≤0.001*
BMI (continuous variable)	1.01	0.98-1.03	0.091	0.99	0.98-1.09	0.110
FIGO stage (III/IV vs I/II)	0.39	0.25-0.62	≤0.001*	0.59	0.47-0.73	≤0.001*
Histologic grade (3 vs 1 & 2)	0.56	0.36-0.89	0.0149	0.99	0.78-1.28	0.991
LVSI (absent vs present)	2.79	1.76-4.42	≤0.001*	1.18	0.99-1.40	0.060
Adjuvant therapy (therapy vs no therapy)	0.85	0.53-1.37	0.51	0.89	0.76-1.05	0.172
MMR status (MMR deficient vs MMR proficient)	1.90	1.34-2.70	0.003*	1.03	0.92-1.18	0.540

*Denotes statistical significance. N=1,514 Endometrioid EC, N=85 deaths during follow-up period, N=58 disease related mortality.

recurrence risk persisted when evaluating patients at low-risk for recurrence by PORTEC criteria (5-year DFS 70.8% vs 95.4%, p=≤0.001). Due to the relatively few patients with early-stage EC and probable MMR mutations who recurred (N=3), we are unable to comment on the effect of probable MMR mutation and recurrence risk.

Discussion

In this study, we confirm and expand on prior reports that epigenetic MMR deficiency in EC is associated with poor prognostic features (9, 24, 38, 39). However, for the first time we demonstrate that MMR deficiency was an independent predictor for lymph node metastasis and recurrence after controlling for these prognostic factors through traditional risk stratification algorithms.

In patients at high risk for LN metastasis by Mayo criteria, EC with epigenetic MMR defect was twice as likely to have LN metastasis (20% vs 10.5%). Tumor size is an established prognostic factor for lymph node involvement and thus has been integrated into risk stratification algorithms used to identify women in whom surgical lymphadenectomy can be safely omitted (23, 25). Indeed, the significantly different rates of lymphadenectomy between MMR deficient tumors (83.7%) and MMR proficient EC (74.1%) illustrates the effect tumor volume may have on surgical decision-making. Our group has previously reported on the association of epigenetic MMR defects with large tumor volume and lymph node metastasis (20) but in this study, we identify that epigenetic MMR defect is associated with lymph node metastasis independent of tumor volume. Recently, Diniz et al. advocated for the use of MMR status to triage patients who require lymphadenectomy (26); however, their analyses did not differentiate between epigenetic loss and those with probable MMR mutation. The association between epigenetic MMR deficits and LN positivity seen in our study suggests that the 22% (15/69) MMR deficient EC with positive LN in their study may largely be attributed to epigenetic MMR deficits.

Factors such as advanced age, higher grade, LVSI, and deeper myometrial invasion are utilized to predict patients at high risk for recurrence despite early stage disease. This study, and others (9, 13, 22, 27, 28), confirms that MMR deficient EC is associated negative prognostic indicators. However, we found that patients with probable MMR mutation did not differ from those with MMR proficient EC in terms of stage, grade, LVSI, and myometrial invasion. Rather, epigenetic MMR defects were the driver for the association of MMR defects with these negative prognostic factors. Given that these prognostic factors are accounted for through risk stratification algorithms (GOG99 and PORTEC) utilized to predict the risk for recurrence and guide adjuvant therapy in early-stage endometrioid EC, we sought to evaluate the role of MMR status after controlling for these factors. We found that there was not a significant difference between MMR proficient EC and EC with probable MMR mutations according to GOG99 and PORTEC risk classification. However, ECs with epigenetic MMR defects were significantly more likely to be classified as high or HIR by traditional risk stratification algorithms; 23.1% vs 10.3% (p=≤0.001) for GOG99 and 23.4% vs 17.8% (p=0.004) for PORTEC criteria. Epigenetic MMR defects were strongly associated with worse DFS in early-stage endometrioid EC independent of risk classification and the receipt of adjuvant therapy.

Finally, when we evaluated all endometrioid ECs, we found that MMR deficiency was independently associated with worse DFS but not OS after controlling for age, BMI, grade, LVSI, stage, and adjuvant therapy.

The association between MMR status and disease recurrence and survival has been extensively studied in a variety of malignancies. Although MMR deficiency has been associated with better prognosis in colorectal cancer its prognostic significance in EC is unclear (29). While many studies have reported worse DFS in patients with MMR deficiency (9, 20, 30, 39) other studies have reported improved outcomes (21, 31, 32, 38). A meta-analysis (33) that sought to evaluate the role of MMR status and clinical outcomes in EC highlights some possible reasons for these discrepancies. Many studies include very small sample sizes (the median sample size in the aforementioned meta-analysis was 112 subjects), heterogeneous patient populations including endometrioid and non-endometrioid histologies, and inconsistent methods for determining and classifying MMR status. Our study has several strengths that we feel empower the findings: (1) The

large sample size of more than 1,500 ECs, (2) only endometrioid histology ECs were isolated to avoid histology as a confounding factor, (3) MMR status was classified using the expression of all 4 MMR proteins, and (4) universal *MLH1* methylation testing was performed. Our study does have some important limitations to address including the relatively modest number of recurrences and the very limited number of recurrences in patients with a probable MMR mutation limits the ability to extrapolate the risk profile in this group. In addition, while the median follow-up period of 2.5 years is relatively short, data has shown that the majority of recurrences will occur within 2 years of initial diagnosis.

Conclusion

Traditional uterine-based risk stratification algorithms may not accurately reflect the risk for lymph node metastasis and recurrence in EC with epigenetic MMR defects. Our findings advocate for the use of molecular classification and MMR testing alongside traditional risk stratification algorithms based on uterine factors. Given the high concordance between MMR IHC status by preoperative biopsy compared to definitive surgical specimen (40–42) these findings also highlight the role of MMR testing on preoperative biopsy specimens to facilitate risk-stratification and patient-centered counseling.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The Ohio State University Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Concept and design (CC, CR, PH). Acquisition, analysis, or interpretation of data (all authors). Drafting of the manuscript (CC, CR). Critical revision of the manuscript for important intellectual content (all authors). Statistical and data analysis (CR, CC). Study supervision (CC, PG, DC). All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1147657/full#supplementary-material

SUPPLEMENTARY FIGURE 1
OS and DFS in early-stage EC by MMR status.

References

1. Backes FJ, Leon ME, Ivanov I, Suarez A, Frankel WL, Hampel H, et al. Prospective evaluation of DNA mismatch repair protein expression in primary endometrial cancer. *Gynecologic Oncol* (2009) 114(3):486–90. doi: 10.1016/j.ygyno.2009.05.026

2. Buchanan DD, Tan YY, Walsh MD, Clendenning M, Metcalf AM, Ferguson K, et al. Tumor mismatch repair immunohistochemistry and DNA MLH1 methylation testing of patients with endometrial cancer diagnosed at age younger than 60 years optimizes triage for population-level germline mismatch repair gene mutation testing. *J Clin Oncol* (2014) 32(2):90–100. doi: 10.1200/JCO.2013.51.2129

3. Lancaster JM, Powell CB, Chen LM, Richardson D and SGO Clinical Practice Committee, et al. Society of gynecologic oncology statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecologic Oncol* (2015) 136(1):3–7. doi: 10.1016/j.ygyno.2014.09.009

4. Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, Chapelle A, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. *Int J Cancer* (1999) 81(2):214–8. doi: 10.1002/(SICI)1097-0215(19990412)81:2<214::AID-IJC8>3.0.CO;2-L

5. Hampel H, Stephens JA, Pukkala E, Sankila R, Aaltonen LA, Mecklin JP, et al. Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: Later age of onset. *Gastroenterology* (2005) 129(2):415–21. doi: 10.1016/j.gastro.2005.05.011

6. Meyer LA, Broaddus RR and Lu KH. Endometrial cancer and lynch syndrome: clinical and pathologic considerations. *Cancer Control* (2009) 16(1):14–22. doi: 10.1177/107327480901600103

7. Lu KH, Dinh M, Kohlmann W, Watson P, Green J, Syngal S, et al. Gynecologic cancer as a "sentinel cancer" for women with hereditary nonpolyposis colorectal cancer syndrome. *Obstet Gynecol* (2005) 105(3):569–74. doi: 10.1097/01.AOG.0000154885.44002.ae

8. Levine DA and the Cancer Genome Atlas Research Network. Integrated genomic characterization of endometrial carcinoma. *Nature* (2013) 497(7447):67–73. doi: 10.1038/nature12113

9. McMeekin DS, Tritchler DL, Cohn DE, Mutch DG, Lankes HA, Geller MA, et al. Clinicopathologic significance of mismatch repair defects in endometrial cancer: An NRG Oncology/Gynecologic oncology group study. *J Clin Oncol* (2016) 34(25):3062–8. doi: 10.1200/JCO.2016.67.8722

10. Yan HHN, Lai JCW, Ho SL, Leung WK, Law WL, Lee JFY, et al. RNF43 germline and somatic mutation in serrated neoplasia pathway and its association with BRAF mutation. *Gut* (2017) 66(9):1645–56. doi: 10.1136/gutjnl-2016-311849

11. Metcalf AM and Spurdle AB. Endometrial tumour BRAF mutations and MLH1 promoter methylation as predictors of germline mismatch repair gene mutation status: a literature review. *Fam Cancer* (2014) 13(1):1–12. doi: 10.1007/s10689-013-9671-6

12. Yan HHM, Lai JCW, Ho SL, Leung WK, Law WL, Lee JFY, et al. RNF43 germline and somatic mutation in serrated neoplasia pathway and its association with BRAF mutation. *Gut* (2017) 66(9):1645–56.

13. Loukovaara M, Pasanen A and Bützow R. Mismatch repair deficiency as a predictive and prognostic biomarker in molecularly classified endometrial carcinoma. *Cancers* (2021) 13(13). doi: 10.3390/cancers13133124

14. Backes FJ, Haag J, Cosgrove CM, Suarez A, Cohn DE, Goodfellow PJ, et al. Mismatch repair deficiency identifies patients with high-intermediate-risk (HIR) endometrioid endometrial cancer at the highest risk of recurrence: A prognostic biomarker. *Cancer* (2019) 125(3):398–405. doi: 10.1002/cncr.31901

15. Njoku K, Barr CE and Crosbie EJ. Current and emerging prognostic biomarkers in endometrial cancer. *Front Oncol* (2022) 12. doi: 10.3389/fonc.2022.890908

16. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* (2017) 357(6349):409–13. doi: 10.1126/science.aan6733

17. Green AK, Feinberg J and Makker V. A review of immune checkpoint blockade therapy in endometrial cancer. *Am Soc Clin Oncol Educ Book* (2020) 40):238–44. doi: 10.1200/EDBK_280503

18. Murali R, Delair DF, Bean SM, Abu-Rustum NR and Soslow RA. Evolving roles of histologic evaluation and Molecular/Genomic profiling in the management of endometrial cancer. *J Natl Compr Canc Netw* (2018) 16(2):201–9. doi: 10.6004/jnccn.2017.7066

19. and National Comprehensive Cancer Network. *Uterine NEoplasms*. (2023) (Version 1.2023). Available at: https://www.nccn.org/professionals/physician_gls/pdf/uterine.pdf.

20. Cosgrove CM, Cohn DE, Hampel H, Frankel WL, Jones D, McElroy JP, et al. Epigenetic silencing of MLH1 in endometrial cancers is associated with larger tumor volume, increased rate of lymph node positivity and reduced recurrence-free survival. *Gynecologic Oncol* (2017) 146(3):588–95. doi: 10.1016/j.ygyno.2017.07.003

21. Fountzilas E, Kotoula V, Pentheroudakis G, Manousou K, Polychronidou G, Vrettou E, et al. Prognostic implications of mismatch repair deficiency in patients with nonmetastatic colorectal and endometrial cancer. *ESMO Open* (2019) 4(2):e000474. doi: 10.1136/esmoopen-2018-000474

22. Backes FJ, Haag J, Cosgrove CM, Suarez A, Cohn DE, Goodfellow PJ, et al. Mismatch repair deficiency identifies patients with high-intermediate–risk (HIR) endometrioid endometrial cancer at the highest risk of recurrence: A prognostic biomarker. *Cancer* (2019) 125(3):398–405. doi: 10.1002/cncr.31901

23. Mariani A, Dowdy SC, Cliby WA, Gostout BS, Jones MB, Wilson TO, et al. Prospective assessment of lymphatic dissemination in endometrial cancer: a paradigm shift in surgical staging. *Gynecol Oncol* (2008) 109(1):11–8. doi: 10.1016/j.ygyno.2008.01.023

24. Bilbao C, Lara PC, Ramirez R, Henriquez-Hernandez LA, Rodriguez F, Falcon O, et al. Microsatellite instability predicts clinical outcome in radiation-treated endometrioid endometrial cancer. *Int J Radiat OncologyBiologyPhysics* (2010) 76 (1):9–13. doi: 10.1016/j.ijrobp.2009.09.035

25. Milam MR, Java J, Walker JL, Metzinger DS, Parker LP, Coleman RL, et al. Nodal metastasis risk in endometrioid endometrial cancer. *Obstetrics Gynecology* (2012) 119(2 Part 1):286–92. doi: 10.1097/AOG.0b013e318240de51

26. Diniz TP, Menezes JN, Goncalves BT, Faloppa CC, Mantoan H, Kumagai LY, et al. Can mismatch repair status be added to sentinel lymph node mapping algorithm in endometrioid endometrial cancer? *Gynecologic Oncol* (2023) 169:131–6. doi: 10.1016/j.ygyno.2022.12.010

27. Kim SR, Pina A, Albert A, McAlpine JN, Wolber R, Gilks B, et al. Mismatch repair deficiency and prognostic significance in patients with low-risk endometrioid endometrial cancers. *Int J Gynecologic Cancer* (2020) 30(6):783–8. doi: 10.1136/ijgc-2019-000910

28. Berg HF, Engerud H, Myrvold M, Lien HE, Hjelmeland ME, Halle MK, et al. Mismatch repair markers in preoperative and operative endometrial cancer samples; expression concordance and prognostic value. *Br J Cancer* (2022). doi: 10.1038/s41416-022-02063-3

29. Popat S, Hubner R and Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* (2005) 23(3):609–18. doi: 10.1200/JCO.2005.01.086

30. Caduff RF, Johnston CM, Svoboda-Newman SM, Poy EL, Merajver SD, Frank TS, et al. Clinical and pathological significance of microsatellite instability in sporadic endometrial carcinoma. *Am J Pathol* (1996) 148(5):1671–8.

31. Maxwell GL, Rinsinger JI, Alvarez AA, Barrett JC and Berchuck A. Favorable survival associated with microsatellite instability in endometrioid endometrial cancers. *Obstet Gynecol* (2001) 97(3):417–22. doi: 10.1016/s0029-7844(00)01165-0

32. Kato M, Takano M, Miyamoto M, Sasaki N, Goto T, Tsuda H, et al. DNA Mismatch repair-related protein loss as a prognostic factor in endometrial cancers. *J Gynecol Oncol* (2015) 26(1):40–5. doi: 10.3802/jgo.2015.26.1.40

33. Diaz-Padilla I, Romero N, Amir E, Matias-Guiu X, Vilar E, Muggia F., et al. Mismatch repair status and clinical outcome in endometrial cancer: a systematic review and meta-analysis. *Crit Rev Oncol Hematol* (2013) 88(1):154–67. doi: 10.1016/j.critrevonc.2013.03.002

34. Keys HM, Roberts JA, Brunetto VL, Zaino RJ, Spirtos NM, Bloss JD, et al. A phase III trial of surgery with or without adjunctive external pelvic radiation therapy in intermediate risk endometrial adenocarcinoma: a gynecologic oncology group study. *Gynecol Oncol* (2004) 92(3):744–51. doi: 10.1016/j.ygyno.2003.11.048

35. Creutzberg CL, Putten WL, Koper PC, Lybeert ML, Jobsen JJ, Warlam-Rodenhuis CC, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC study group. post operative radiation therapy in endometrial carcinoma. *Lancet* (2000) 355(9213):1404–11. doi: 10.1016/S0140-6736(00)02139-5

36. Nout RA, Smit VTHBM, Putter H, Jurgenliemk-Schulz IM, Jobsen JJ, Lutgens LCHW, et al. Vaginal brachytherapy versus pelvic external beam radiotherapy for patients with endometrial cancer of high-intermediate risk (PORTEC-2): an open-label, non-inferiority, randomised trial. *Lancet* (2010) 375(9717):816–23. doi: 10.1016/S0140-6736(09)62163-2

37. Mariani A, Webb MJ, Keeney GL, Haddock MG, Calori G, Podratz KC, et al. Low-risk corpus cancer: is lymphadenectomy or radiotherapy necessary? *Am J Obstet Gynecol* (2000) 182(6):1506–19. doi: 10.1067/mob.2000.107335

38. Black D, Soslow RA, Levine DA, Tornos C, Chen SC, Hummer AJ, et al. Clinicopathologic significance of defective DNA mismatch repair in endometrial carcinoma. *J Clin Oncol* (2006) 24(11):1745–53. doi: 10.1200/JCO.2005.04.1574

39. Cohn DE, Frankel WL, Resnick KE, Zanagnolo VL, Copeland LJ, Hampel H, et al. Improved survival with an intact DNA mismatch repair system in endometrial cancer. *Obstetrics Gynecology* (2006) 108(5):1208–15. doi: 10.1097/01.AOG.0000239097.42987.0c

40. Stelloo E, Nout RA, Naves LCLM, Ter Haar NT, Creutzberg CL, Smit VTHBM, et al. High concordance of molecular tumor alterations between pre-operative curettage and hysterectomy specimens in patients with endometrial carcinoma. *Gynecologic Oncol* (2014) 133(2):197–204. doi: 10.1016/j.ygyno.2014.02.012

41. Abdulfatah E, Wakeling E, Sakr S, Al-Abaidy K, Bandyopadhyay S, Morris R, et al. Molecular classification of endometrial carcinoma applied to endometrial biopsy specimens: Towards early personalized patient management. *Gynecologic Oncol* (2019) 154(3):467–74. doi: 10.1016/j.ygyno.2019.06.012

42. Chapel DB, Yamada SD, Cowan M and Lastra R. Immunohistochemistry for mismatch repair protein deficiency in endometrioid endometrial carcinoma yields equivalent results when performed on endometrial biopsy/curettage or hysterectomy specimens. *Gynecologic Oncol* (2018) 149(3):570–4. doi: 10.1016/j.ygyno.2018.04.005

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 18 April 2023
DOI 10.3389/fonc.2023.1147591

Check for updates

OPEN ACCESS

EDITED BY
Raheleh Rahbari,
Wellcome Sanger Institute (WT),
United Kingdom

REVIEWED BY
Joseph Christopher,
University of Cambridge, United Kingdom
Zongming (Eric) Chen,
Mayo Clinic, United States

*CORRESPONDENCE
Maria Rasmussen
maria.rasmussen.07@regionh.dk

SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 18 January 2023
ACCEPTED 23 March 2023
PUBLISHED 18 April 2023

CITATION
Rasmussen M, Sowter P, Gallon R, Durhuus JA, Hayes C, Andersen O, Nilbert M, Schejbel L, Høgdall E, Santibanez-Koref M, Jackson MS, Burn J and Therkildsen C (2023) Mismatch repair deficiency testing in Lynch syndrome-associated urothelial tumors. *Front. Oncol.* 13:1147591. doi: 10.3389/fonc.2023.1147591

COPYRIGHT
© 2023 Rasmussen, Sowter, Gallon, Durhuus, Hayes, Andersen, Nilbert, Schejbel, Høgdall, Santibanez-Koref, Jackson, Burn and Therkildsen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Mismatch repair deficiency testing in Lynch syndrome-associated urothelial tumors

Maria Rasmussen[1*], Peter Sowter[2], Richard Gallon[2], Jon Ambæk Durhuus[1,3], Christine Hayes[2], Ove Andersen[1], Mef Nilbert[4], Lone Schejbel[5], Estrid Høgdall[5], Mauro Santibanez-Koref[6], Michael S. Jackson[6], John Burn[2] and Christina Therkildsen[7]

[1]Department of Clinical Research, Copenhagen University Hospital - Amager and Hvidovre, Copenhagen, Denmark, [2]Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom, [3]Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark, [4] Institute of Clinical Sciences, Division of Oncology and Pathology, Lund University, Lund, Sweden, [5]Molecular Unit, Department of Pathology, Copenhagen University Hospital – Herlev and Gentofte, Copenhagen, Denmark, [6]Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom, [7]The Danish HNPCC Register, Gastro Unit, Copenhagen University Hospital - Amager and Hvidovre, Copenhagen, Denmark

Introduction: Lynch syndrome-associated cancer develops due to germline pathogenic variants in one of the mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6* or *PMS2*. Somatic second hits in tumors cause MMR deficiency, testing for which is used to screen for Lynch syndrome in colorectal cancer and to guide selection for immunotherapy. Both MMR protein immunohistochemistry and microsatellite instability (MSI) analysis can be used. However, concordance between methods can vary for different tumor types. Therefore, we aimed to compare methods of MMR deficiency testing in Lynch syndrome-associated urothelial cancers.

Methods: Ninety-seven urothelial (61 upper tract and 28 bladder) tumors diagnosed from 1980 to 2017 in carriers of Lynch syndrome-associated pathogenic MMR variants and their first-degree relatives (FDR) were analyzed by MMR protein immunohistochemistry, the MSI Analysis System v1.2 (Promega), and an amplicon sequencing-based MSI assay. Two sets of MSI markers were used in sequencing-based MSI analysis: a panel of 24 and 54 markers developed for colorectal cancer and blood MSI analysis, respectively.

Results: Among the 97 urothelial tumors, 86 (88.7%) showed immunohistochemical MMR loss and 68 were successfully analyzed by the Promega MSI assay, of which 48 (70.6%) were MSI-high and 20 (29.4%) were MSI-low/microsatellite stable. Seventy-two samples had sufficient DNA for the sequencing-based MSI assay, of which 55 (76.4%) and 61 (84.7%) scored as MSI-high using the 24-marker and 54-marker panels, respectively. The concordance between the MSI assays and immunohistochemistry was 70.6% (p = 0.003), 87.5% (p = 0.039), and 90.3% (p = 1.00) for the Promega assay, the 24-marker assay, and the 54-marker assay, respectively. Of the 11 tumors with retained MMR protein expression, four were MSI-low/MSI-high or MSI-high by the Promega assay or one of the sequencing-based assays.

Conclusion: Our results show that Lynch syndrome-associated urothelial cancers frequently had loss of MMR protein expression. The Promega MSI assay was significantly less sensitive, but the 54-marker sequencing-based MSI analysis showed no significant difference compared to immunohistochemistry. Data from this study alongside previous studies, suggest that universal MMR deficiency testing of newly diagnosed urothelial cancers, using immunohistochemistry and/or sequencing-based MSI analysis of sensitive markers, offer a potentially useful approach to identification of Lynch syndrome cases.

KEYWORDS

Lynch syndrome, urothelial cancer, universal testing, mismatch repair deficiency, immunohistochemistry, microsatellite instability

1 Introduction

The cancer-predisposition syndrome, Lynch syndrome, is caused by pathogenic germline variants in the mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*¸ or *PMS2*. Beside a high risk of colorectal and endometrial cancer, Lynch syndrome has been linked to an increased risk of both upper (ureteral and renal pelvic) and lower (bladder) urothelial cancer (1–4), with urothelial cancers being the third most common cancer in this population. The highest risk of urothelial cancer is observed in patients with pathogenic germline variants in *MSH2* with cumulative risk estimates at age 70 of 5.8–6.9% for upper urinary tract (ureter and renal pelvis) cancer and 2.6–12.3% for bladder cancer, compared to 2.2–4.8% and 0–10.8% for those harboring *MLH1* pathogenic variants and 0–2.9% and 0–1.7% for those harboring *MSH6* pathogenic variants for upper urinary tract and bladder cancers, respectively (2, 4). The cumulative risk of urothelial cancer at age 70, can be as high as 25.5% for men (4).

Loss of MMR function is an early event in most Lynch syndrome tumors as a single hit in the still functioning MMR allele can induce total loss of function. The resulting MMR deficiency causes an excessive accumulation of mutations especially in small repetitive DNA sequences, referred to as microsatellite instability (MSI). As loss of MMR protein expression and/or MSI are found in most Lynch syndrome-associated cancers, these analyses can be used as screening tools to increase identification of Lynch syndrome individuals and facilitate cancer-preventive surveillance strategies for affected patients and their family members. In addition, loss of MMR protein expression and MSI analyses have gained increased clinical interest during recent years, as these biomarkers were approved by the American Food and Drug Administration (FDA) in 2017 to guide immunotherapy with pembrolizumab across tumor types (5, 6).

MMR deficiency in tumors can for most cases be visualized by either immunohistochemical staining using antibodies directed against the four MMR proteins to show loss of expression, or by MSI analyses that assess changes in the length of microsatellites by PCR and fragment length analysis (typically capillary electrophoresis) or sequencing-based methods (7–10). Immunohistochemical analyses can be difficult to interpret due to intra- and inter-observer variability or lack of internal positive control cells and they are insensitive to MMR missense variants that disrupt function whilst retaining protein expression. Therefore, a combination of the two tools has been proposed (11–13). A standardized panel of five MSI markers (BAT-25, BAT-26, NR-21, NR-24, and MONO-27), which comprise the MSI Analysis System v1.2 (Promega), has been widely used during the last two decades towards identification of Lynch syndrome colorectal cancer patients (14). However, the Promega MSI assay may be less sensitive for MMR deficiency than immunohistochemistry when used in extra-colorectal cancers (15, 16). Furthermore, this method classifies tumors into three disjunct categories: MSI-high (MSH-H), MSI-low (MSI-L), and microsatellite stable (MSS), where MSI-H indicates MMR deficiency. However, MSI classification can be sensitive to the number and identity of MSI markers investigated, and the biological reality is likely a gradient from MSS to MSI-H rather than categorical subsets (17–19).

New methods to assess MSI have consequently been developed that use alternative methods, markers, and automated and dichotomized classifiers (20–24). For example, the Idylla™ MSI Test uses PCR and high resolution melt curve analysis of seven alternative markers, and has shown promising results for colorectal cancer, though the concordance with immunohistochemical analyses was lower for some endometrial cancers (25, 26). Assessment of MSI status using next generation sequencing of microsatellites captured within targeted panel, exome, or genome sequencing can be achieved using a variety of classifiers. Three such classifiers, MSIsensor, mSINGS, and MANTIS, were tested in six types of cancer, including colorectal, endometrial, esophagus, gastric, and prostate cancers, and achieved a concordance of 77%–100% when compared to the Bethesda MSI assay using PCR and fragment length analyses of three dinucleotide and two mononucleotide markers (20). Unfortunately, no urothelial tumors were analyzed in this study.

We have previously tested the Promega MSI assay in a small cohort of Lynch syndrome-associated urothelial cancers and found only 23% of the tumors to be MSI-H while 90% had immunohistochemical loss of MMR proteins (2), suggesting that MSI analysis of urothelial cancers may not be a suitable screen for Lynch syndrome. A gene panel sequencing-based analysis that classified 551 unselected urothelial cancers using MSIsensor found that 5.8% had increased MSI, and that 37.5% of these were Lynch syndrome-associated (27), demonstrating the potential clinical utility of a different MSI analysis method. This study was, however, conducted in an anonymized cohort, and immunohistochemical analyses of the MSS Lynch syndrome-associated cancers were not possible, meaning that false-negatives may have been missed and concordance between these two methods was not established. Hence, additional studies for immunohistochemical and MSI analyses for urothelial cancers are warranted.

A single molecule molecular inversion probe (smMIP), amplicon sequencing-based MSI assay of 24-markers previously achieved 100% sensitivity and 100% specificity in over 200 colorectal cancers compared to the Promega MSI assay (28, 29). Very recently, this assay has been further enhanced using a panel of 54 markers selected for instability in the normal peripheral blood of patients with the childhood cancer syndrome constitutional MMR deficiency (CMMRD). These novel 54 markers allowed much greater separation of CMMRD blood samples from controls than was achieved with the original 24 markers, and similarly improved MSI classification of colorectal cancers (30), indicating they may have utility in other tumor types as well. Here, we compare immunohistochemical MMR loss in an updated cohort of 97 Lynch syndrome-associated urothelial tumors to a variety of MSI assays including the Promega MSI Analysis v1.2 and the smMIP amplicon sequencing-based approach using both the 24-marker and 54-marker panels.

2 Materials and methods

2.1 Study population and samples

Individuals with surgically removed urothelial (ureteral, renal pelvic and/or bladder) cancer diagnosed between January 1st, 1980, to December 19th, 2017, with either a verified pathogenic germline variant in one of the MMR genes, *MLH1*, *MSH2*, *MSH6*, and *PMS2*, or being a first-degree relative (FDR) to such were included. The individuals were identified through the Danish Hereditary Non-Polyposis Colorectal Cancer (HNPCC) Register, and formalin-fixed paraffin-embedded tumor tissues were identified and collected from Pathology Departments around Denmark. Thirty-six of the cases have been investigated in a previous study (2).

Clinical data corresponding to the collected tumor specimen including genetic MMR variant, family relation, sex, tumor site, age at diagnosis, surgery date, tumor stage and differentiation grade were extracted from the HNPCC register.

The study was approved by the Scientific and Ethics Committee of the Capital Region of Copenhagen, Denmark (H-17001916) and the Data Protection Agency (AHH-2017-071).

2.2 Immunohistochemical staining of mismatch repair proteins

Immunohistochemical analyses of the MMR proteins on whole slides were available for 36 of the tumors and have been previously published (2). The remaining tumors (N=61) were analyzed at the Department of Pathology, Herlev Hospital, with ready-to-use antibodies against MLH1 (clone ES05, Agilent, California, USA), MSH2 (clone FE11, Agilent), MSH6 (clone EP49, Agilent), and PMS2 (clone EP51, Agilent) using the Dako PT link machine (Agilent) with high pH antigen retrieval buffer for pretreatment and the Dako Omnis (Agilent) and the EnVision FLEX, high pH, kit (Agilent) according to manufacturer's instructions. The immunohistochemical analyses were performed on tissue microarrays (containing 2 × 1 mm biopsies from tumor areas located using Hematoxylin and Eosin whole slide staining of each tumor) or 4 µm thin slices for 8 of the tumors.

All immunohistochemical stained slides were scanned and evaluated using the NDP.view2 viewing software (Hamamatsu Photonics K.K., Shizuoka, Japan) by two independent observers (MR and CT). Loss of MLH1, MSH2, MSH6, and PMS2 was defined as protein expression in ≤10% of the tumor cells in the presence of internal positive staining in control cells e.g., lymphocytes or stromal cells. This arbitrary cut-off was used as immunohistochemical screening of colorectal and endometrial cancer has shown that half of the cases with <10% positive tumor cells can be explained by pathogenic MMR germline mutations (31). This approach does not consider subclonal loss of MMR expression among tumor cells. We did, however, not find subclonal MMR loss in any of the samples studied. Only one sample was difficult to score with 30% positive tumor cells. This tumor was scored as positive according to the scoring criteria above but is mentioned in detail in the results.

2.3 DNA extraction

DNA was extracted from formalin-fixated paraffin-embedded tumor samples using the QIAamp® DNA FFPE Tissue kit (QIAGEN, Germany). Samples were either processed as 10 µm sections, macro-dissected sections, or cores (1 mm in diameter), depending on the amount of tumor cells within the block and the thickness of the block. Sections and macro-dissected samples were incubated in 320 µL deparaffinization solution (QIAGEN), vortexed, incubated at 56°C for 3 minutes, cooled to room temperature (RT) and centrifuged at 11,000 x g for 1 minute. However, core samples were treated twice with 1ml xylene (Histolab, Sweden) for 30-60 minutes and centrifuged for 2 minutes at max speed in between and after. Pellet was resuspended in 99.5% ethanol and incubated for 30-60 minutes, supernatant was removed, and pellet was dried for 20 minutes. 180 µL ATL buffer (QIAGEN) was added, and the tubes were centrifuged at 11,000 x g for 1 minute. Subsequently, 20 µL proteinase K (QIAGEN) was added to the clear phase and incubated at 56°C overnight on an orbital shaker at 650 RPM. If lysis was not complete, additional 20 µL proteinase K was added. The samples were then incubated at 90°C for a maximum of 60

minutes, centrifuged, and the clear phase was transferred to new tubes to which 2 units of AmpErase™ uracil N-glycosylase (UNG) (Thermo Fisher Scientific, Massachusetts, USA) was added. Samples were vortexed, centrifuged, and incubated at 50°C for 60 minutes, and subsequently centrifuged and incubated for 2 minutes at room temperature with 2 µL RNase A (QIAGEN). The rest of the DNA extraction was carried out on an automated QiaCube (QIAGEN) platform as described by the manufacturer and eluted in 40 µL distilled water.

DNA concentrations were measured using Qubit dsDNA BR kit and fluorometer 3.0 (Invitrogen, Massachusetts, USA) to determine the volume needed for the MSI analyses. Only samples with double stranded DNA concentrations above 1 ng/µL could be analyzed for MSI-status, resulting in exclusion of 2 samples.

2.4 Microsatellite analysis using Promega MSI analysis system version 1.2

MSI analysis using the Promega MSI Analysis System v1.2, including mononucleotide repeat markers *BAT-25*, *BAT-26*, *NR-21*, *NR-24*, and *MONO-27* plus two control pentanucleotide repeat markers to distinguish between samples and detect possible contamination (14). This analysis was performed according to the manufacturer's protocol, however without use of matched normal DNA. PCR amplification used a Verity 96 thermocycler (Applied Biosystem, Massachusetts, USA). DNA fragments were separated by capillary electrophoresis on the 3130*xl* Genetic Analyzer (Applied Biosystem, Massachusetts, USA) and data was analyzed with GeneMapper Software 5 (Thermo Fisher Scientific, Massachusetts, USA) using AFLP default analysis settings. Interpretation was done by manual visual inspection of electropherogram traces by two experts in molecular biology (LS and EH). Due to the lack of matched normal samples, only samples with three or more unstable markers were classified as MSI-H (32). Samples with no unstable markers were classified as MSS. Samples with one unstable marker were classified as unresolved MSS/MSI-L, while samples with two unstable markers were classified as unresolved MSI-L/MSI-H. Samples with one or more markers with traces suggestive of instability that could not be confirmed due to lack of paired normal tissue were considered non-evaluable. To evaluate concordance with immunohistochemistry, MSI-L/MSI-H was pooled with MSI-H and referred to as pooled MSI-H, while MSS/MSI-L was pooled with MSS and referred to as pooled MSS/MSI-L.

2.5 Sequencing-based microsatellite instability analysis

2.5.1 MSI markers and probe pooling, and phosphorylation

The MSI markers used in the smMIP amplicon sequencing-based MSI approach were mononucleotide repeats selected in previously published studies. Two panels were tested: A panel of 24 mononucleotide repeats selected based on their instability in sequence data from colorectal cancers (28, 29), and a panel of 54 markers selected based on their instability in whole genome sequencing data of normal (non-neoplastic) blood from patients with CMMRD (30). smMIPs to capture the MSI markers are described in the above-mentioned studies. All the probes were pooled, 5'-phosphorylated, and diluted to 0.1 nM per probe as previously described (28, 33).

2.5.2 Probe target capture and amplification

MSI markers were probe-captured and amplified in multiplex using the previously described protocol (28, 33) using 23-273ng of sample DNA and a SensoQuest thermocycler. In brief, the targeting arms of the probes were annealed to the sample DNA template, the gap between the arms filled by a high-fidelity polymerase, and the 3' end ligated to the 5' end of the probe, resulting in circularized products. Linear DNA (sample DNA and excess probes) was degraded by exonucleases. Finally, the circularized probes containing the regions of interest were amplified by conventional PCR using universal primers that amplify from the common probe backbone sequence. Amplicons were analyzed by capillary electrophoresis using a QIAxcel (Qiagen, Germany) and the AL420 program, expecting amplicons in the range of 222–287 base-pairs (bp).

2.5.3 Library preparation and sequencing

Amplicons were purified by Agencourt AMPure XP beads (Beckman Coulter, Indianapolis, USA) and quantified by Qubit dsDNA HS Kit (Invitrogen) using a Qubit Fluorometer 3.0 (Invitrogen). Purified amplicons with a dsDNA concentration of at least 1.89 ng/µL (12nM) were diluted to 4 nM in 10 mM Tris, pH 8.5, and then pooled in equal volumes to create the sequencing library. The library was sequenced using a 12 pM loading concentration and MiSeq v3 Kit (Illumina, California, USA) on a MiSeq platform (Illumina) following manufacturer's protocols. The Generate FASTQ workflow, paired-end sequencing, and custom sequencing primers were used as previously described (28, 33) to a target depth of >2000 reads per amplicon per sample.

2.5.4 Bioinformatic analysis and MSI classification

Microsatellite variant detection and MSI classification using the frequency and allelic bias of microsatellite deletions and a naïve Bayesian approach has been described previously (29). Published data from a cohort of 50 MSI-H and 52 MSS colorectal cancers analyzed by both the 24-marker and 54-marker assays and the same smMIP amplicon sequencing-based protocol (30) was used to train the MSI classifier. An MSI score <0 classified a sample as MSS, while a score >0 classified a sample as MSI-H.

2.6 Statistical analyses

Clinical data and results of MSI and immunohistochemical analyses were imported into R (34) in which all statistical analyses

were performed. Comparison of the characteristics for carriers of germline pathogenic variants and FDRs were performed with Fisher's exact test for categorical variables, while Welch Two Sample t-test was used to compare the mean age. Concordance between the immunohistochemical analysis and each MSI assay was calculated as the frequency of matched samples, by matching retained MMR protein expression with pooled MSS/MSI-L and loss of MMR protein expression with pooled MSI-H, divided by the total amount of successfully analyzed samples. Concordance was tested using the Exact McNemar test while the confidence intervals were calculated using Wilson confidence intervals. Concordance was visualized using ggplot2 package (35). Specificity and sensitivity for each method were calculated using the epiR package in R (36). The Exact McNemar test was also used to compare the two smMIP sequencing-based panels. To investigate the association between explanatory factors, such as tumor location, age of the archival material, MMR gene, DNA quantity, and whether a sample could be evaluated by MSI analysis, Fisher's exact test was used for categorical variables while the Kruskal-Wallis Rank Sum Test was used for continuous variables. All statistical analyses were two-tailed and statistical significance was reached when $p < 0.05$. Truncating variants as defined here include frameshift, splice-site, large deletions, and non-sense variants.

3 Results

3.1 Cohort characteristics

In total, 97 tumors resected from verified carriers or FDRs of known carriers of germline pathogenic variants in *MSH2* (66.0%), *MSH6* (23.7%), *MLH1* (9.3%), or *PMS2* (1.1%), were retrospectively collected and analyzed (Table 1). The mean age at diagnosis was 63.9 years and the majority of the tumors developed in women (59.8%). The tumor location was equally distributed between bladder tumors (including one ureteric orifice) (37.1%), renal pelvic tumors (35.1%), and ureteral tumors (27.8%) (Table 1). *MSH2* was more frequently affected in carriers, while *MSH6* was almost as frequently affected as *MSH2* in FDRs ($p < 0.001$) (Table 1). There were no significant differences in sex, age, or tumor location between carriers and FDRs (Table 1). Ten of the included patients developed two or more synchronous tumors, while five patients developed metachronous tumors, and one developed both synchronous and metachronous tumors.

3.2 Loss of mismatch repair protein expression

Of the 97 tumors, 86 (88.7%) showed loss of MMR protein expression, all correlating to the MMR gene affected in the respective family (Supplementary Table 1). MMR protein loss was more frequent in carriers, with 69 out of 74 (93.2%) showing MMR protein loss, compared to 17 out of 23 (73.9%) FDRs ($p = 0.020$), suggesting that the FDR group may include non-carriers. The five MMR proficient tumors from carriers were observed in carriers of *MSH2* (N=1) and *MSH6* (N=4). One of the four *MSH6* variants was a missense variant (c.3259C>T), which has been associated with low levels of MSI, suggesting it might have lost MMR function while retaining MMR protein expression in a subset of the tumor cells (30%). We found a significant difference between upper and lower tract urothelial cancer and

TABLE 1 Clinical data of Lynch syndrome-associated urothelial cancers.

Characteristics	All urothelial cancers	Cancers from carriers	Cancers from FDRs	P-value
Age at diagnosis (range and mean)	63.9 (31–89)	62.8 (42–82)	67.09 (31–89)	0.189
Sex, N (%)	**N=97**	**N=74**	**N=23**	0.089
Male	39 (40.2%)	26 (35.1%)	13 (56.5%)	
Female	58 (59.8%)	48 (64.9%)	10 (43.5%)	
Genes, N (%)	**N=97**	**N=74**	**N=23**	<0.001
MLH1	9 (9.3%)	7 (9.5%)	2 (8.7%)	
MSH2	64 (66.0%)	53 (71.6%)	11 (47.8%)	
MSH6	23 (23.7%)	14 (18.9%)	9 (39.1%)	
PMS2	1 (1.1%)	0 (0%)	1 (4.3%)	
Location, N (%)	**N=97**	**N=74**	**N=23**	0.912
Ureter	27 (27.8)	21 (28.4%)	6 (26.1%)	
Renal pelvis	34 (35.1)	25 (33.8%)	9 (39.1%)	
Bladder (including ureteric orifice)	36 (37.1)	28 (37.8%)	8 (34.8%)	

10.3389/fonc.2023.1147591

MMR protein expression, with nine of the 11 tumors with retained MMR protein expression being in the lower urothelial tract (p = 0.002) (Table 2). When dividing the cohort by carrier status, tumor location was significantly associated with immunohistochemical MMR protein expression only for the FDRs: Five of the six FDR tumors and four of the five carrier tumors with retained MMR protein expression were in the lower urinary tract (p = 0.009 and p = 0.065, respectively). This suggest that some of the lower urothelial tract cancers might be due to sporadic origin and not Lynch syndrome.

TABLE 2 Concordance between immunohistochemical analysis and the three MSI analyses divided by carrier status and tumor location.

		Total	Promega*	24-marker	54-marker
Carriers		N	N (%)	N (%)	N (%)
Ureter/renal pelvis	**MMR protein loss**	**45**			
	MSI		25 (56%)	30 (67%)	33 (73%)
	MSS		8 (18%)	5 (11%)	2 (4%)
	NE		12 (27%)	10 (22%)	10 (22%)
	Retained MMR protein	**1**			
	MSI		1 (100%)	0 (0%)	0 (0%)
	MSS		0 (0%)	1 (100%)	1 (100%)
	NE		0 (0%)	0 (0%)	0 (0%)
Bladder	**MMR protein loss**	**24**			
	MSI		13 (54%)	15 (63%)	15 (63%)
	MSS		5 (21%)	1 (4%)	1 (4%)
	NE		6 (25%)	8 (33%)	8 (33%)
	Retained MMR protein	**4**			
	MSI		1 (25%)	1 (25%)	2 (50%)
	MSS		1 (25%)	3 (75%)	2 (50%)
	NE		2 (50%)	0 (0%)	0 (0%)
FDR		N	N (%)	N (%)	N (%)
Ureter/renal pelvis	**MMR protein loss**	**14**			
	MSI		6 (43%)	8 (57%)	9 (64%)
	MSS		3 (21%)	2 (14%)	1 (7%)
	NE		5 (36%)	4 (29%)	5 (36%)
	Retained MMR protein	**1**			
	MSI		0 (0%)	0 (0%)	0 (0%)
	MSS		0 (0%)	1 (100%)	1 (100%)
	NE		1 (100%)	0 (0%)	0 (0%)
Bladder	**MMR protein loss**	**3**			
	MSI		1 (33%)	1 (33%)	1 (33%)
	MSS		1 (33%)	0 (0%)	0 (0%)
	NE		1 (33%)	2 (67%)	2 (67%)
	Retained MMR protein	**5**			
	MSI		1 (20%)	1 (20%)	1 (20%)
	MSS		2 (40%)	4 (80%)	3 (60%)
	NE		2 (40%)	1 (20%)	1 (20%)

*For readability the Promega assay score "MSI-L/MSI-H" was categorized as "MSI", while "MSS/MSI-L" was categorized as "MSS".

3.3 Microsatellite instability analysis using promega MSI analysis system v1.2 and concordance with immunohistochemistry

The Promega assay gave interpretable results for 68 of the tumors (70.1%) of which 36 (52.9%) were MSI-H, 12 (17.6%) were MSI-L/MSI-H, 12 (17.6%) were MSS/MSI-L, and eight (11.8%) were MSS (Supplementary Table 1). Immunohistochemical MMR protein expression and Promega MSI analyses were concordant for 48 of the samples (70.6%) (95% CI 58.8–80.1%, p = 0.003) (Figure 1). Assuming that the immunohistochemical analysis is the reference method for identification of cases with underlying germline pathogenic MMR variants, the Promega MSI assay could identify Lynch syndrome cases with a sensitivity of 72.6% (95% CI 59.8–83.1%) and a specificity of 50.0% (95% CI 11.8–88.2%).

Investigating the cases with retained MMR protein expression and MSI status, we found three tumors with retained expression that were MSI-H (N=1) or MSI-L/MSI-H (N=2) (Supplementary Table 1, Table 2). A possible explanation for this discordance could be missense variants, leading to a non-functional MMR protein but retained MMR protein expression. However, all three cases (two carriers and one FDR) were associated with germline truncating variants.

Investigating the MSS cases with loss of immunohistochemical MMR protein expression, we found 17 samples that were MSS or MSS/MSI-L and had loss of immunohistochemical expression of MMR proteins. Thirteen of these were from carriers, including one carrier of a truncating variant in *MLH1*, seven carriers of a variant in *MSH2* with six known to be truncating and the seventh not having a type reported, and five carriers of truncating variants in *MSH6* (Supplementary Table 1, Table 2). For the Promega MSI analyses, there was no statistically significant correlation between MSI status (using pooled classification, see Materials and methods) and tumor location (p = 0.415).

3.4 Microsatellite instability analysis using smMIP amplicon sequencing

The smMIP amplicon sequencing-based MSI assay was successfully performed for 72 of the included tumors (75.6%) of which 62 (86.1%) had loss of MMR protein expression. The 24-marker assay classified 55 of the tumors as MSI-H (76.4%), while the 54-marker assay identified six additional tumors as MSI-H (N=61, 84.7%) (p = 0.031) (Figure 1). A comparison between the 24-marker assay and immunohistochemical MMR protein expression showed that all but one of the 55 tumors that were MSI-H had loss of MMR protein expression, while nine out of the 17 MSS tumors had retained MMR protein expression

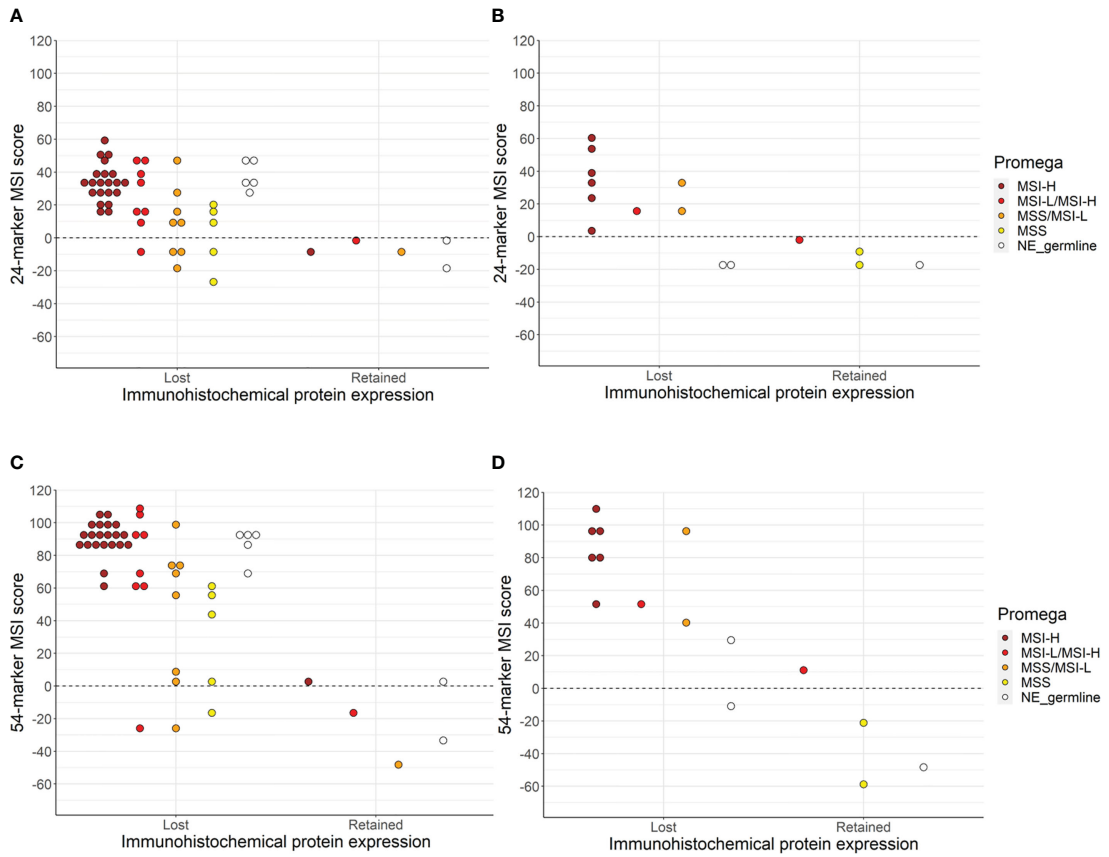


FIGURE 1
The concordance between immunohistochemical protein expression, Promega MSI assay, and sequenced-based MSI assay using the 24-marker assay for carriers **(A)**, and FDRs **(B)** or the 54-marker assay for carriers **(C)** and FDRs **(D)**. NE_germline are the samples that were non-evaluable (NE) due to the lack of germline DNA.

(Supplementary Table 1, Table 2). This gave a concordance of 87.5% (95% CI 77.6–93.4%, p = 0.039), sensitivity of 87.1% (95% CI 76.1–94.3%), and specificity of 90% (95% CI 55.5–99.7%) for the 24-marker assay compared to immunohistochemical analyses. The one tumor with retained MMR protein expression that was MSI-H was from a carrier with a missense *MSH6* variant (c.3259C>T), which showed loss of MSH6 protein expression in 70% of the tumor cells. This sample was not evaluable in the Promega assay due to lack of germline DNA. The eight MSS tumors with immunohistochemical protein loss developed in six carriers and two FDRs. Of the six carriers, one had a truncating *MSH2* variant, while the remaining five had truncating *MSH6* variants. There was no significant association between MSI status and upper versus lower urothelial cancer (p = 0.253).

For the new 54-marker panel, three of the 61 MSI-H tumors had retained MMR protein expression, while seven of the 11 MSS samples showed retained MMR protein expression (Supplementary Table 1, Table 2). This gave a concordance of 90.3% (95% CI 80.9–95.4%, p = 1), a sensitivity of 93.5% (95% CI 84.3–98.2%), and a specificity of 70% (95% CI 34.8–93.3%) for the 54-marker sequencing-based MSI assay relative to immunohistochemical analysis. Retained MMR protein expression was found for three MSI-H samples, in an *MSH6* carrier with a truncating variant, an *MSH6* carrier with a missense variant (this was the same tumors as for the 24-marker assay), and an FDR with a truncating *MSH2* variant. Of the 11 MSS tumors, three carriers and one FDR had loss of MMR protein expression. Of the carriers, two had a truncating *MSH6* variant and one had a truncating *MSH2* variant. Again, there was no significant association between MSI status and tumor location (p = 0.173). Overall, for the 54-marker assay, an increase in the separation of the sample scores was seen compared to the 24-marker assay with a mean score of 58.6 (range -58.9–109.9) compared to 19.9 (range -26.9–60.4).

3.5 Discordance between the two sequencing-based assays

Of the six samples classified as MSS by the 24-marker panel but MSI-H by the 54-marker panel, five had low positive scores ranging from 0.7–11.1 (Table 3). Four belonged to carriers, all having truncating *MSH6* variants. One of these had retained expression of MSH6 but was MSI-H when analyzed by the Promega MSI assay. The three remaining *MSH6* carriers all had loss of MSH6 protein expression, and one was MSS and two were MSS/MSI-L when analyzed by the Promega assay. The remaining two cases developed in FDRs, one belonging to a family with a truncating variant in *MSH2* and this individual's tumor had retained MSH2 expression but was MSI-L/MSI-H when analyzed by the Promega assay. The other FDR belonged to a family with a truncating *MSH6* variant, and the tumor showed loss of expression of MSH6. The Promega data was inconclusive due to missing germline DNA. This sample had the largest change in MSI score, from -17.6 for the 24-marker assay to 29.5 for the 54-marker assay. Together, these observations suggest the 54-marker assay may be more sensitive than the 24-marker assay.

3.6 Non-evaluable samples for the MSI assays

Twenty-nine tumors were non-evaluable by the Promega MSI assay due to technical issues such as low signal intensity or failed amplification (N=18), or due to uncertain results that could not be determined without germline material for comparison (N=11). Samples being non-evaluable due to technical issues was significantly associated with the age of the tumor blocks (p = 0.004), but not with DNA concentration (p = 0.644). For the sequencing-based assays, 25 samples were excluded from sequencing, fourteen of which were also non-evaluable by the Promega assay. The excluded samples were again from significantly older tumor blocks (p < 0.001) but were not associated with DNA concentration (p = 0.476).

4 Discussion

Testing for tumor MMR deficiency has dual clinical functions - both as a screening tool to identify Lynch syndrome patients and to guide use of immune checkpoint blockade therapy, especially within colorectal cancer. Both immunohistochemical protein expression and MSI analyses can be used to investigate MMR deficiency. However, these methods have primarily been developed for and used in colorectal cancers. Limited data is available for extra-colonic cancers, including urothelial cancers, especially comparing the two methods. Molecular studies investigating urothelial cancer primarily aim to screen for

TABLE 3 Overview of the six samples that were discordant with the 24- and 54- marker panels.

Carrier status	Gene	MMR protein immunohisto-chemistry	Promega MSI assay classification	24-marker assay MSI score	54-marker assay MSI score
FDR	MSH2	Retained	MSI-L/MSI-H	-1.9 (MSS)	11.1 (MSI-H)
Carrier	MSH6	Retained	MSI-H	-7.3 (MSS)	3.8 (MSI-H)
FDR	MSH6	Lost	NE_germline	-17.6 (MSS)	29.5 (MSI-H)
Carrier	MSH6	Lost	MSS/MSI-L	-10.3 (MSS)	4.7 (MSI-H)
Carrier	MSH6	Lost	MSS/MSI-L	-7.7 (MSS)	8.7 (MSI-H)
Carrier	MSH6	Lost	MSS	-26.9 (MSS)	0.7 (MSI-H)

Lynch syndrome patients in large, unselected cohorts using immunohistochemistry and/or MSI analysis and germline MMR test a selected subgroup. Thus only few Lynch syndrome cases are included and often Lynch syndrome tumors with retained MMR protein and/MSS tumors will not be MMR germline tested (37, 38). Hence, the proportion of Lynch syndrome patients with retained immunohistochemical MMR protein expression and/or MSS status remains uncertain. In this study, we tested a large and updated cohort of 97 Lynch syndrome-associated urothelial cancer, to our knowledge the largest cohort of its type to be tested for MMR deficiency. Using immunohistochemical analyses of MMR protein expression, we found 88.7% had lost MMR protein expression in accordance with the gene affected within the family. This is in accordance with smaller studies of Lynch syndrome-associated urothelial cancer, in which 82-100% of the tumors showed loss of MMR protein expression (4, 39). In contrast, a previous study of upper tract urothelial cancer, reported loss of immunohistochemical MMR protein expression in 30% of the patients with a common Lynch syndrome-associated cancer, including patients with no verified Lynch syndrome-associated pathogenic MMR variant, and it was not possible to extract the numbers from verified Lynch syndrome individuals only (40). This study also included FDRs who might be non-carriers, explaining some of the tumors without loss of MMR protein expression. During the final preparation of this manuscript, one of the included FDRs was found to be a non-carrier from a recent gene test. This individual was, however, kept in the study as molecular and statistical analyses had already been performed and since MMR proficient samples are valuable for the concordance analyses and are scarce in the study cohort. Considering only carriers, 93.2% had immunohistochemical loss of MMR protein expression. This correlates well with what previous studies found for other Lynch syndrome-associated extra-colorectal cancers (41–44). In summary, these data indicate that immunohistochemical MMR protein expression can be used to identify Lynch syndrome cancers but does not identify all. We found an association between retained MMR protein expression and lower urinary tract cancers, which might be explained by lower tract urinary cancers are more common in the general population than upper tract urinary cancers and Lynch syndrome individuals can also develop sporadic cancers, i.e., not due to their underlying MMR germline variant (45). MMR immunohistochemistry has limitations since missense MMR variants might not be identified with immunohistochemistry and the evaluations are subjective (11,1213). Hence, objective evaluations, also giving a dichotomized evaluation, could be warranted.

We have previously analyzed a small cohort of Lynch syndrome urothelial cancer and shown that the conventional Promega MSI method had difficulties identifying all the MMR protein deficient tumors (2) (36 of the tumors included in this study). In this updated and larger cohort, the Promega assay found 52.9% of the tumors to be MSI-H and 17.6% to be MSI-L/MSI-H (pooled MSI-H = 70.5%). We also tested a new sequencing-based method with two different MSI marker sets, one previously derived from colorectal cancer sequence data (28), and the other containing MSI markers derived from whole genome sequencing of normal (non-neoplastic) blood of individuals with CMMRD syndrome, which was found to be more sensitive than the 24-marker panel also for colorectal cancers (30). The 24-marker assay found 76.4% to be MSI-H, while the new 54-marker assay found 84.7% to be MSI-H. These results suggest that MSI analyses have potential to identify Lynch syndrome cancers, but careful marker selection and validation may be required.

For the 54-marker assay, we also observed a wider range of scores. This and its increased sensitivity can, in part, be explained by the larger number of markers analyzed compared to the 24-marker assay. However, in the study in which they were identified it was shown that these new MSI markers were individually more sensitive than the original 24-marker panel for both constitutional and tumor analyses (30). It was evident that five of the tumors, that were MSS in the original panel gave a low score above zero, since they are close to the threshold separating MSS and MSI-H. These five could potentially be false positives in the 54-marker assay. However, as four of these were from *MSH6* carriers and one FDR from an *MSH6* family this might explain the low scores, since they generally were lower for *MHS6* (30). If the samples were false negatives in the 24-marker assays this could be explained by subclonal MMR deficiency, where the MSI signal is just below the limit of detection. False negatives/positives could also be explained by the MSI classifier being trained using colorectal cancers and for future studies it would be pertinent to re-train the MSI classifier on urothelial cancers.

Difference in the MSI-H proportion have been observed for Lynch syndrome cancer depending on the specific organ. A previous study analyzing MSI in Lynch syndrome-associated tumors using the Bethesda MSI panel, which includes mononucleotide and dinucleotide repeat markers, found that most of the ureteral tumors were MSI-H whilst the frequency was lower for bladder cancer, kidney cancer, and brain cancer (39). Likewise, MSIsensor found varying frequencies of samples being MSI-H in different cancer types (27). We did not find a difference between upper and lower urothelial cancers and the MSI status for the three MSI assays, only for immunohistochemical MMR protein expression. This was only significant for the FDR cohort but not the carrier cohort, albeit a similar trend was observed. There was a correlation between lower tract urothelial tumors and retained MMR protein expression. This suggests that the tumors might be sporadic and not due to the underlying germline MMR variant. This is consistent with lower tract urothelial cancer being more common in the general population (45).

The concordance between the MSI assays and immunohistochemical MMR protein expression was highest for the 54-marker assay (90.3%) and lowest for the Promega assay (70.6%). A systematic review of universal screening of upper tract urothelial cancer, found a concordance of 58.7% (range 40%-100%) between immunohistochemical MMR protein expression and (primarily) the Bethesda panel (38). In total, 57 samples gave evaluable results by all three MSI assays, of which the sequencing-based method performed better than the Promega MSI assay. According to immunohistochemical MMR protein expression, only 39 tumors showed concordance using the Promega assay, while 51 samples were concordant using the 24-marker assay, and 52 tumors using the 54-marker assay. Exome and genome sequencing increase the potential number of

MSI markers to analyze. However, increasing the number of MSI markers analyzed may not increase sensitivity and specificity as assay accuracy also depends on the sensitivity and specificity of the individual MSI markers. Indeed, assay sensitivity and specificity can be maintained or even improved with smaller, select MSI marker panels (20, 21, 28, 30). MSI and immunohistochemical MMR protein expression have been used interchangeably but based on this study the two types of analyses does not always identify the same samples. However, MSI can identify a few additional samples that does not show immunohistochemical loss so it could be used in addition to immunohistochemistry to identify as many tumors with MMR deficiency as possible. A recent review of the diagnostic yield of universal immunohistochemical testing has shown that this test identifies up to 4.7% individuals with Lynch syndrome (36). The screening was, however, designed in a way that only MMR deficient tumors were referred to MSI testing and/or genetic analyses, which results in the lack of identification of Lynch syndrome tumors that are MMR proficient but MSI.

In addition, Lynch syndrome-associated extra-colorectal cancers have been found to have a lower concordance between MSI and immunohistochemistry MMR protein expression. In a study of Lynch syndrome-associated ovarian cancer, six patients were tested for both immunohistochemical loss and Promega MSI with four being MSI-H giving a concordance of 66.7% (15). In a study in our group we found a concordance between Idylla™ MSI and immunohistochemical loss of 60.0% for Lynch syndrome-associated cancer (43), while concordance was 96.4% for immunohistochemical protein expression and Idylla™ MSI for colorectal cancer (46). For Lynch syndrome-associated endometrial cancer, immunohistochemical loss identified 100% while MSI-H with Promega were only identified in 56% of the investigated tissues (16). The lower concordance for extra-colorectal cancers, might also suggest that other markers than the ones used in the Promega assay might be more characteristic for MSI status in other tumor types. As mentioned above, MSI status was not significantly different between upper and lower tract urothelial cancers for any of the MSI assays used in this study. Furthermore, all of the sequencing-based markers were intergenic, whereas Promega had both intronic and exonic markers (47), which might affect their alterations differently depending on organ. Other reasons might be due to timing of MMR deficiency with immunohistochemical loss as an initial step followed by MSI at a later stage. This could be analyzed by TNM stage and MSI status as the number of altered microsatellite sequences are likely to increase during tumor development. However, we did not investigate this due to lack of complete TNM data.

The MSI status from both types of assays did not correlate completely with the immunohistochemical staining and we found tumors with retained MMR protein expression showing MSI. One reason for this, could be that retained immunohistochemical protein expression does not necessarily mean that the MMR-protein is functional. It has been found that Lynch syndrome-associated colorectal tumors express MMR proteins despite having a missense germline variant to a higher degree than truncating variants (11). These tumors have been found to be MSI-H (48, 49). In here, we found four tumors that were MSI-H but with retained MMR-proteins with either of the two types of assays but only one of these cases could be explained by a germline *MSH6* missense variant, in which 30% of the tumor cells expressed all MMR proteins. Additional reasons could be unknown genetic or epigenetic mutations affecting the MMR system such as the somatic second hit being a missense variant. In addition, it is well-known that immunohistochemical MMR protein expression analyses does not identify loss in all Lynch syndrome-associated tumors (7, 8, 50).

In this study, it was not possible to analyze all the samples for MSI, and of the 29 (29.9%) non-evaluable samples for the Promega assay and the 25 (25.8%) samples excluded from the sequenced-based MSI analyses, 14 (14.4%) could not be evaluated by either assay. Although formalin fixation and paraffin embedding is an efficient way to store tumor tissues it can lead to DNA degradation, hence, lower DNA quality and quantity, which will lead to PCR errors such as stutter bands in the electropherogram traces and less amplification product (51, 52). In this cohort 82.5% (N=80) of the tumors were surgically removed in 1980-2011, hence they are more than 10 years old. The non-evaluable samples were significantly older than the evaluable samples, but no significant difference was found regarding DNA concentration or MMR gene. In this study, all 97 samples gave evaluable results when analyzed with immunohistochemical MMR protein analyses; hence, this may be the best method in a retrospective setting. However, the two methods might complement each other.

In contrast to the Promega assay, the MSI classification used by the sequencing-based assay only focuses on deletions, since similar levels of insertions in mononucleotide repeats were found for both MSI and MSS samples but it differed for deletions (29). A limitation of the Promega assay, is the longer monomeric sequences as these may lead to PCR artifacts (53) that can affect the evaluation and thereby interpretation of MSI status. In addition, the fragment lengths are evaluated manually which may induce subjectivity. Our interpretation of Promega results were challenged by the lack of matched normal samples to check for germline polymorphisms and minor shifts in fragment lengths. Indeed, some of our urothelial cancer samples showed more subtle changes in mononucleotide lengths were found compared to the larger shifts we usually see for colorectal cancers, which has also been observed for endometrial cancer (54). This may lead to classification of fewer MSI-H cancers. To circumvent this, we subdivided the MSI groups even further, adding MSI-L/MSI-H and MSS/MSI-L, for the tumors that with two or one unstable markers, respectively. Of the 12 MSI-L/MSI-H tumors, nine were categorized as MSI-H using the 54-marker assay, indicating that inclusion of germline DNA might have improved the Promega results. In the new version of the Promega MSI kit, OncoMate™ MSI Dx Analysis System, recommendations for MSI analyses have been included and only deviations of three or more bp should now be considered MSI, just as it was stated previously by Suraweera (32). In addition, the new kit can also be used with automated software and these initiatives can lead to less subjective evaluations, although it still needs to be validated prior to implementation. The sequencing-based MSI classification used in this study is automatic, however, it is currently only trained for

colorectal cancers which might lead to incorrect evaluation for other cancer types. Studies in a prospective setting are warranted and further validation is needed prior to clinical use.

Another consideration is the use of software that detect MSI and other mutational signatures of MMR deficiency alongside detection of MMR gene variants in gene panel, exome, and genome sequence data. Software like MMRDetect, MSIdetect, MSISensor, mSINGS, and MANTIS have demonstrated high accuracies (>95%) for the detection of MSI/MMR deficiency in a variety of tumor types (10, 20–22). Therefore, as these sequencing methods are increasingly used for diagnostic and treatment purposes, it is possible that targeted MSI analysis will become redundant. However, targeted MSI analysis is lower cost, generates significantly less data, and can allow faster turnaround times. Therefore, it will likely be a useful method for some time, particularly in low to middle-income countries.

5 Conclusion

We found that immunohistochemical analyses identified loss of MMR protein expression in 88.7% of the Lynch syndrome-associated urothelial tumors analyzed. Comparing the MSI assays with immunohistochemical analyses, Promega was concordant for 70.6%, the 24-marker assay for 87.5%, and the 54-marker assay for 90.3%. We did not find a statistical difference between immunohistochemistry and the 54-marker panel. However, as each method identified few unique MMR deficient cases, we recommend using both immunohistochemistry of MMR proteins and the 54-marker panel for a truly comprehensive test for both screening purposes and treatment with immune checkpoint inhibitors. This is a retrospective study performed on archival samples which should be validated in a prospective setting prior to clinical implementation.

Data availability statement

The FASTQ files generated for the sequence-based MSI assay are available at the European Nucleotide Archive with the following study identification: PRJEB58093 (https://www.ebi.ac.uk/ena/browser/view/PRJEB58093).

Ethics statement

The studies involving human participants were reviewed and approved by Scientific and Ethics Committee of the Capital Region of Copenhagen, Denmark (HD-2007-0032 and H-17001916). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization: MR, PS, RG, MJ, JB, CT. Methodology: MR, PS, RG, JD, LS, EH, CH. Software: MR, PS, RG. Validation: MR, PS, RG, LS, EH. Formal analyses: MR, PS, RG, MS-K. Investigation: MR, PS, RG, CT. Resources: OA, MN, PS, EH, MJ, JB, CT. Data curation: MR. Writing – original draft: MR, CT. Writing – review and editing: MR, PS, RG, JD, CH, OA, MN, LS, EH, MS-K, MJ, JB, CT. Visualization: MR, RG. Supervision: RG, PS, MN, CT. Project administration: MR, PS, RG. Funding acquisition: MR, MN, CT. All authors contributed to the article and approved the submitted version.

Funding

This study was funded by the Danish Cancer Society Research Center (A15946 and A14570) including the salary for MR. The Swedish Cancer Society provided funding used for DNA extraction. PS and RG were funded by the Aspirin for Cancer Prevention (AsCaP) group, Cancer Research UK Grant Code: C569/A24991.

Acknowledgments

The authors would like to thank Mats Jönsson and Eva Rambech for their help with DNA extraction at Skåne University Hospital Cancer Center, Lund, Sweden. In addition, we would like to thank the Department of Pathology, Copenhagen University Hospital – Herlev and Gentofte, Copenhagen, Denmark, for their help with immunohistochemical stainings and Promega MSI analyses, especially Christina Grønhøj, Dorte Skriver-Jensen, and Alma Dedic. We would also like to thank Thomas Kallemose, Department of Clinical Research, Copenhagen University Hospital - Amager and Hvidovre Copenhagen, Denmark, for statistical discussions.

Conflict of interest

JB, MJ, and MS-K are named inventors on a patent covering the 24 microsatellite instability marker panel analyzed with the following patent ID: WO/2018/037231 published March 1, 2018. RG, JB, MJ, and MS-K are inventors and CH is a contributor of a patent covering a minimal set of the 24 microsatellite instability marker panel with the following patent ID: WO/2021/019197 published February 4, 2021. RG, JB, MJ, and MS-K are named inventors on a patent covering the 54 microsatellite instability marker panel analyzed with the following patent ID: GB2114136.1 filed October 1, 2021.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1147591/full#supplementary-material

SUPPLEMENTARY TABLE 1
An overview of the immunohistochemistry, MSI analyses and relevant clinical data.

References

1. Dominguez-Valentin M, Sampson JR, Seppälä TT, ten Broeke SW, Plazzer JP, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the prospective lynch syndrome database. *Genet Med* (2020) 22(1):15–25. doi: 10.1038/s41436-019-0596-9

2. Joost P, Therkildsen C, Dominguez-Valentin M, Jönsson M, Nilbert M. Urinary tract cancer in lynch syndrome; increased risk in carriers of MSH2 mutations. *Urology* (2015) 86(6):1212–7. doi: 10.1016/j.urology.2015.08.018

3. Skeldon SC, Semotiuk K, Aronson M, Holter S, Gallinger S, Pollett A, et al. Patients with lynch syndrome mismatch repair gene mutations are at higher risk for not only upper tract urothelial cancer but also bladder cancer. *Eur Urol* (2013) 63(2):379–85. doi: 10.1016/j.eururo.2012.07.047

4. van der Post RS, Kiemeney LA, Ligtenberg MJL, Witjes JA, Hulsbergen-van de Kaa CA, Bodmer D, et al. Risk of urothelial bladder cancer in lynch syndrome is increased, in particular among MSH2 mutation carriers. *J Med Genet* (2010) 47(7):464–70. doi: 10.1136/jmg.2010.076992

5. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* (2017) 357(6349):409–13. doi: 10.1126/science.aan6733

6. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite Instability/Mismatch repair-deficient cancer: Results from the phase II KEYNOTE-158 study. *J Clin Oncol* (2020) 38(1):1–10. doi: 10.1200/JCO.19.02105

7. Hampel H, Pearlman R, Beightol M, Zhao W, Jones D, Frankel WL, 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 (6):806–13. doi: 10.1001/jamaoncol.2018.0104

8. Hampel H, Pearlman R, de la Chapelle A, Pritchard CC, Zhao W, Jones D, et al. Double somatic mismatch repair gene pathogenic variants as common as lynch syndrome among endometrial cancer patients. *Gynecol Oncol* (2021) 160(1):161–8. doi: 10.1016/j.ygyno.2020.10.012

9. Li K, Huang L, Luo H, Zhu X. Microsatellite instability: A review of what the oncologist should know. *Cancer Cell Int* (2020) 20(1):16. doi: 10.1186/s12935-019-1091-8

10. Zou X, Koh GCC, Nanda AS, Degasperi A, Urgo K, Roumeliotis TI, et al. A systematic CRISPR screen defines mutational mechanisms underpinning signatures caused by replication errors and endogenous DNA damage. *Nat Cancer* (2021) 2 (6):643–57. doi: 10.1038/s43018-021-00200-0

11. Chen W, Hampel H, Pearlman R, Jones D, Zhao W, Alsomali M, et al. Unexpected expression of mismatch repair protein is more commonly seen with pathogenic missense than with other mutations in lynch syndrome. *Hum Pathol* (2020), 103:34–41. doi: 10.1016/j.humpath.2020.07.001

12. Klarskov L, Ladelund S, Holck S, Roenlund K, Lindebjerg J, Elebro J, et al. Interobserver variability in the evaluation of mismatch repair protein immunostaining. *Hum Pathol* (2010) 41(10):1387–96. doi: 10.1016/j.humpath.2010.03.003

13. Overbeek LIH, Ligtenberg MJL, Willems RW, Hermens RPMG, Blokx WAM, Dubois SV, et al. Interpretation of immunohistochemistry for mismatch repair proteins is only reliable in a specialized setting. *Am J Surg Pathol* (2008) 32(8):1246–51. doi: 10.1097/PAS.0b013e31816401bb

14. Bacher JW, Flanagan LA, Smalley RL, Nassif NA, Burgart LJ, Halberg RB, et al. Development of a fluorescent multiplex assay for detection of MSI-high tumors. *Dis Markers* (2004) 20(4–5):237–50. doi: 10.1155/2004/136734

15. Crosbie EJ, Ryan NAJ, McVey RJ, Lalloo F, Bowers N, Green K, et al. Assessment of mismatch repair deficiency in ovarian cancer. *J Med Genet* (2020) 58(10):687–91. doi: 10.1136/jmedgenet-2020-107270

16. Ryan NAJ, McMahon R, Tobi S, Snowsill T, Esquibel S, Wallace AJ, et al. The proportion of endometrial tumours associated with lynch syndrome (PETALS): A prospective cross-sectional study. *PloS Med* (2020) 17(9):e1003263. doi: 10.1371/journal.pmed.1003263

17. Tomlinson I, Halford S, Aaltonen L, Hawkins N, Ward R. Does MSI-low exist? *J Pathol* (2002) 197(1):6–13. doi: 10.1002/path.1071

18. Verma L, Kane MF, Brassett C, Schmeits J, Evans DG, Kolodner RD, et al. Mononucleotide microsatellite instability and germline MSH6 mutation analysis in early onset colorectal cancer. *J Med Genet* (1999) 36(9):678–82.

19. You JF, Buhard O, Ligtenberg MJL, Kets CM, Niessen RC, Hofstra RMW, et al. Tumours with loss of MSH6 expression are MSI-h when screened with a pentaplex of five mononucleotide repeats. *Br J Cancer* (2010) 103(12):1840–5. doi: 10.1038/sj.bjc.6605988

20. Kautto EA, Bonneville R, Miya J, Yu L, Krook MA, Reeser JW, et al. Performance evaluation for rapid detection of pan-cancer microsatellite instability with MANTIS. *Oncotarget* (2017) 8(5):7452–63. doi: 10.18632/oncotarget.13918

21. Marques AC, Ferraro-Peyret C, Michaud F, Song L, Smith E, Fabre G, et al. Improved NGS-based detection of microsatellite instability using tumor-only data. *Front Oncol* (2022) 12:969238. doi: 10.3389/fonc.2022.969238

22. Niu B, Ye K, Zhang Q, Lu C, Xie M, McLellan MD, et al. MSIsensor: microsatellite instability detection using paired tumor-normal sequence data. *Bioinformatics* (2014) 30(7):1015–6. doi: 10.1093/bioinformatics/btt755

23. Pécriaux A, Favre L, Calderaro J, Charpy C, Derman J, Pujals A. Detection of microsatellite instability in a panel of solid tumours with the idylla MSI test using extracted DNA. *J Clin Pathol* (2021) 74(1):36–42. doi: 10.1136/jclinpath-2020-206581

24. Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC. Microsatellite instability detection by next generation sequencing. *Clin Chem* (2014) 60(9):1192–9. doi: 10.1373/clinchem.2014.223677

25. Dedeurwaerdere F, Claes KB, Van Dorpe J, Rottiers I, van der Meulen J, Breyne J, et al. Comparison of microsatellite instability detection by immunohistochemistry and molecular techniques in colorectal and endometrial cancer. *Sci Rep* (2021) 11 (1):12880. doi: 10.1038/s41598-021-91974-x

26. Gilson P, Levy J, Rouyer M, Demange J, Husson M, Bonnet C, et al. Evaluation of 3 molecular-based assays for microsatellite instability detection in formalin-fixed tissues of patients with endometrial and colorectal cancers. *Sci Rep* (2020) 10(1):16386. doi: 10.1038/s41598-020-73421-5

27. Latham A, Srinivasan P, Kemel Y, Shia J, Bandlamudi C, Mandelker D, et al. Microsatellite instability is associated with the presence of lynch syndrome pan-cancer. *J Clin Oncol* (2019) 37(4):286–95. doi: 10.1200/JCO.18.00283

28. Gallon R, Sheth H, Hayes C, Redford L, Alhilal G, O'Brien O, et al. Sequencing-based microsatellite instability testing using as few as six markers for high-throughput clinical diagnostics. *Hum Mutat* (2020) 41(1):332–41. doi: 10.1002/humu.23906

29. Redford L, Alhilal G, Needham S, O'Brien O, Coaker J, Tyson J, et al. A novel panel of short mononucleotide repeats linked to informative polymorphisms enabling effective high volume low cost discrimination between mismatch repair deficient and proficient tumours. *PloS One* (2018) 13(8):e0203052. doi: 10.1371/journal.pone.0203052

30. Gallon R, Phelps R, Hayes C, Brugieres L, Guerrini-Rousseau L, Colas C, et al. Constitutional microsatellite instability, genotype, and phenotype correlations in constitutional mismatch repair deficiency. *Gastroenterology* (2022). 164(4):579–92.e8. doi: 10.1053/j.gastro.2022.12.017

31. Sarode VR, Robinson L. Screening for lynch syndrome by immunohistochemistry of mismatch repair proteins: Significance of indeterminate result and correlation with mutational studies. *Arch Pathol Lab Med* (2019) 143:1225–33. doi: 10.5858/arpa.2018-0201-OA

32. Suraweera N, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, et al. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. *Gastroenterology* (2002) 123(6):1804–11. doi: 10.1053/gast.2002.37070

33. Hiatt JB, Pritchard CC, Salipante SJ, O'Roak BJ, Shendure J. Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation. *Genome Res* (2013) 23(5):843–54. doi: 10.1101/gr.147686.112

34. R Core Team. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing (2023). Available at: https://www.R-project.org/.

35. Wickham H. *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag (2016). Available at: https://ggplot2.tidyverse.org.

36. Carstensen B, Plummer M, Laara E, Hills M. *Epi: A package for statistical analysis in epidemiology* (2022). Available at: https://CRAN.R-project.org/package=Epi.

37. Gallon R, Gawthorpe P, Phelps RL, Hayes C, Borthwick GM, Santibanez-Koref M, et al. How should we test for lynch syndrome? a review of current guidelines and future strategies. *Cancers (Basel)* (2021) 13(3):406. doi: 10.3390/cancers13030406

38. Rasmussen M, Madsen MG, Therkildsen C. Immunohistochemical screening of upper tract urothelial carcinomas for lynch syndrome diagnostics: a systematic review. *Urology* (2022). 165:44–53. doi: 10.1016/j.urology.2022.02.006

39. Gylling AHS, Nieminen TT, Abdel-Rahman WM, Nuorva K, Juhola M, Joensuu EI, et al. Differential cancer predisposition in lynch syndrome: insights from molecular analysis of brain and urinary tract tumors. *Carcinogenesis* (2008) 29(7):1351–9. doi: 10.1093/carcin/bgn133

40. Harper HL, McKenney JK, Heald B, Stephenson A, Campbell SC, Plesec T, et al. Upper tract urothelial carcinomas: frequency of association with mismatch repair protein loss and lynch syndrome. *Mod Pathol* (2017) 30(1):146–56. doi: 10.1038/modpathol.2016.171

41. Dominguez-Valentin M, Joost P, Therkildsen C, Jonsson M, Rambech E, Nilbert M. Frequent mismatch-repair defects link prostate cancer to lynch syndrome. *BMC Urol* (2016) 16:15. doi: 10.1186/s12894-016-0130-1

42. Nilbert M, Therkildsen C, Nissen A, Akerman M, Bernstein I. Sarcomas associated with hereditary nonpolyposis colorectal cancer: broad anatomical and morphological spectrum. *Fam Cancer* (2009) 8(3):209–13. doi: 10.1007/s10689-008-9230-8

43. Rasmussen M, Lim K, Rambech E, Andersen MH, Svane IM, Andersen O, et al. Lynch syndrome-associated epithelial ovarian cancer and its immunological profile. *Gynecol Oncol* (2021) 162(3):686–93. doi: 10.1016/j.ygyno.2021.07.001

44. Therkildsen C, Ladelund S, Rambech E, Persson A, Petersen A, Nilbert M. Glioblastomas, astrocytomas and oligodendrogliomas linked to lynch syndrome. *Eur J Neurol* (2015) 22(4):717–24. doi: 10.1111/ene.12647

45. Rouprêt M, Babjuk M, Compérat E, Zigeuner R, Sylvester RJ, Burger M, et al. European Association of urology guidelines on upper urinary tract urothelial carcinoma: 2017 update. *Eur Urol* (2018) 73(1):111–22. doi: 10.1016/j.eururo.2017.07.036

46. Velasco A, Tokat F, Bonde J, Trim N, Bauer E, Meeney A, et al. Multi-center real-world comparison of the fully automated Idylla™ microsatellite instability assay with routine molecular methods and immunohistochemistry on formalin-fixed paraffin-embedded tissue of colorectal cancer. *Virchows Arch* (2021) 478(5):851–63. doi: 10.1007/s00428-020-02962-x

47. Hempelmann JA, Lockwood CM, Konnick EQ, Schweizer MT, Antonarakis ES, Lotan TL, et al. Microsatellite instability in prostate cancer by PCR or next-generation sequencing. *J ImmunoTher Cancer* (2018) 6(1):29. doi: 10.1186/s40425-018-0341-y

48. Thibodeau SN, French AJ, Roche PC, Cunningham JM, Tester DJ, Lindor NM, et al. Altered expression of hMSH2 and hMLH1 in tumors with microsatellite instability and genetic alterations in mismatch repair genes. *Cancer Res* (1996) 56 (21):4836–40.

49. Wahlberg SS, Schmeits J, Thomas G, Loda M, Garber J, Syngal S, et al. Evaluation of microsatellite instability and immunohistochemistry for the prediction of germ-line MSH2 and MLH1 mutations in hereditary nonpolyposis colon cancer families. *Cancer Res* (2002) 62(12):3485–92.

50. Hechtman JF, Rana S, Middha S, Stadler ZK, Latham A, Benayed R, et al. Retained mismatch repair protein expression occurs in approximately 6% of microsatellite instability-high cancers and is associated with missense mutations in mismatch repair genes. *Mod Pathol* (2020) 33(5):871–9. doi: 10.1038/s41379-019-0414-6

51. Guyard A, Boyez A, Pujals A, Robe C, Tran Van Nhieu J, Allory Y, et al. DNA Degrades during storage in formalin-fixed and paraffin-embedded tissue blocks. *Virchows Arch* (2017) 471(4):491–500. doi: 10.1007/s00428-017-2213-0

52. Sieben NLG, Ter Haar NT, Cornelisse CJ, Jan Fleuren G, Cleton-Jansensen AM. PCR artifacts in LOH and MSI analysis of microdissected tumor cells. *Hum Pathol* (2000) 31(11):1414–9. doi: 10.1016/S0046-8177(00)80013-1

53. Fazekas A, Steeves R, Newmaster S. Improving sequencing quality from PCR products containing long mononucleotide repeats. *Biotechniques* (2010) 48(4):277–85. doi: 10.2144/000113369

54. Wang Y, Shi C, Eisenberg R, Vnencak-Jones CL. Differences in microsatellite instability profiles between endometrioid and colorectal cancers. *J Mol Diagn* (2017) 19 (1):57–64. doi: 10.1016/j.jmoldx.2016.07.008

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 24 April 2023
DOI 10.3389/fonc.2023.1146825

Check for updates

OPEN ACCESS

EDITED BY
Toni T. Seppälä,
Helsinki University Central Hospital, Finland

REVIEWED BY
Andrew Latchford,
St. Mark's Hospital, United Kingdom
Lars Lindberg,
Hvidovre Hospital, Denmark

*CORRESPONDENCE
Eduardo Vilar
evilar@mdanderson.org

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Cancer Genetics, a section of the journal Frontiers in Oncology

RECEIVED 17 January 2023
ACCEPTED 11 April 2023
PUBLISHED 24 April 2023

CITATION
del Carmen G, Reyes-Uribe L, Goyco D, Evans K, Bowen CM, Kinnison JL, Sepeda VO, Weber DM, Moskowitz J, Mork ME, Thirumurthi S, Lynch PM, Rodriguez-Bigas MA, Taggart MW, You YN and Vilar E (2023) Colorectal surveillance outcomes from an institutional longitudinal cohort of lynch syndrome carriers. *Front. Oncol.* 13:1146825. doi: 10.3389/fonc.2023.1146825

COPYRIGHT
© 2023 del Carmen, Reyes-Uribe, Goyco, Evans, Bowen, Kinnison, Sepeda, Weber, Moskowitz, Mork, Thirumurthi, Lynch, Rodriguez-Bigas, Taggart, You and Vilar. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Colorectal surveillance outcomes from an institutional longitudinal cohort of lynch syndrome carriers

Gabriel del Carmen[1,2†], Laura Reyes-Uribe[1†], Daniel Goyco[1,3], Kyera Evans[1], Charles M. Bowen[1], Jennifer L. Kinnison[1], Valerie O. Sepeda[1], Diane M. Weber[1], Julie Moskowitz[4], Maureen E. Mork[4], Selvi Thirumurthi[4,5], Patrick M. Lynch[4,5], Miguel A. Rodriguez-Bigas[4,6], Melissa W. Taggart[7], Y. Nancy You[4,6] and Eduardo Vilar[1,4*]

[1]Department of Clinical Cancer Prevention, The University of Texas MD Anderson Cancer Center, Houston, TX, United States, [2]McGovern Medical School, University of Texas Health Science Center, Houston, TX, United States, [3]Department of Medicine, Baylor College of Medicine, Houston, TX, United States, [4]Clinical Cancer Genetics Program, The University of Texas MD Anderson Cancer Center, Houston, TX, United States, [5]Department of Gastroenterology, Hepatology and Nutrition, The University of Texas MD Anderson Cancer Center, Houston, TX, United States, [6]Department of Colorectal Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX, United States, [7]Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX, United States

Objective: Lynch Syndrome (LS) carriers have a significantly increased risk of developing colorectal cancer (CRC) during their lifetimes. Further stratification of this patient population may help in identifying additional risk factors that predispose to colorectal carcinogenesis. In most LS patients CRC may arise from adenomas, although an alternative non-polypoid carcinogenesis pathway has been proposed for *PMS2* carriers. Using data from our institutional LS cohort, our aim was to describe our current colorectal screening outcomes with a focus on the incidence of adenomas in the context of different MMR genotypes and patient demographics such as gender, race, and ethnicity.

Design: We collected demographics, genetic, colonoscopy, and pathology results from a total of 163 LS carriers who obtained regular screening care at MD Anderson Cancer Center. Data were extracted from the electronic health records into a REDCap database for analysis. Logistic regressions were performed to measure the association between MMR variants and the likelihood of adenomas, advanced adenomas, and CRC. Then, we analyzed the cumulative incidences of these outcomes for the first 36 months following enrollment using Kaplan-Meier incidence curves, and Cox proportional hazard regressions.

Results: On multivariate analysis, age (≥45 years old) was associated with an increased risk of developing adenomas (P=0.034). Patients with a prior or active cancer status were less likely to develop adenomas (P=0.015), despite of the lack of association between surgical history with this outcome (P=0.868). We found no statistically significant difference in likelihood of adenoma development

between *MLH1* and *MSH2/EPCAM, MSH6, and PMS2* carriers. Moreover, we observed no statistically significant difference in the likelihood of advanced adenomas or CRC for any measured covariates. On Cox proportional hazard, compared to *MLH1* carriers, the incidence of adenomas was highest among *MSH2/EPCAM* carriers during for the first 36-months of follow-up ($P<0.001$). We observed a non-statistically significant trend for Hispanics having a higher and earlier cumulative incidence of adenomas compared to non-Hispanics ($P=0.073$). No MMR carrier was more likely to develop advanced adenomas. No difference in the incidence of CRC by MMR gene ($P=0.198$).

Conclusion: Screening recommendations for CRC in LS patients should be based on specific MMR variants and should also be tailored to consider patient demographics.

KEYWORDS

lynch, colorectal < cancer type, surveillance, colonoscopy, premalignancies

Introduction

Lynch syndrome (LS) is a genetic condition associated with an increased risk of developing multiple types of cancers and it is best known for being the most frequent cause of inherited colorectal and endometrial cancers (1, 2). Colorectal cancer (CRC) is the third most common cancer by incidence worldwide and the second by mortality among all cancers (3). While the general population has an approximately 5% life-time cumulative risk of developing CRC, LS carriers have estimated risks between 10% and 50% depending on the mismatch repair (MMR) gene (4–6). LS carcinogenesis is secondary to alterations in the mismatch repair (MMR) system, which corrects base-pairing errors that occur during DNA replication (7). More specifically, LS results from constitutional variations in one of the four MMR genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) or deletion within *EPCAM*, which promotes hypermethylation and silences *MSH2* (7). Given the heightened risk of the development of CRC for this patient population, adequate colonoscopy screening intervals are crucial for the identification and subsequent removal of pre-cancers (i.e., adenomas). Projected yearly transition rates from advanced adenomas to carcinomas range between 2.6 to 5.6% in the general population, with age being the most significant risk factor (8, 9). The transition rate is estimated to be even higher in the LS cohort, although the true transition rate is unknown; therefore, this population requires more frequent colonoscopies (10) with current recommendations on the age to start screening and frequency intervals based on specific gene variants. In fact, *MLH1, MSH2*, and *EPCAM* carriers are advised to initiate screening at age 20-25 or, if diagnosed before age 25, 2-5 years before the earliest diagnosis of CRC in the family, with intervals every 1-2 years (11–13). In contrast, screening should start later and be performed less frequently among *MSH6* and *PMS2* carriers with the first colonoscopy at age 30-35 or, if diagnosed before age 30, 2-5 years prior to a familial CRC diagnosis, with intervals every 1-3 years (11). With a focus on gene variants, the role of patient demographics, particularly the contribution of race and ethnicity, has not been appropriately addressed in the current recommendations. This omission might be significant since it has already been documented that racial and ethnic minorities are often referred for genetic testing at a diminished rate despite universal screening and genetic testing recommendations (14). Therefore, there is an unmet need to optimize CRC screening in this specific population of patients.

Here, we report the colonoscopy findings from a cohort of LS participants from a single institution. This longitudinal dataset allow us to characterize and report colorectal screening outcomes. In making this information available, our goal is to stimulate a re-evaluation of current cancer surveillance recommendations as well as to contribute to an understanding of how patient demographics affect colorectal adenoma and CRC development in the LS population.

Materials and methods

Study design and participants

A total of 163 LS patients were recruited to an IRB-approved protocol (MDACC IRB# PA12-0327) between March 2013 and March 2020 at the University of Texas MD Anderson Cancer Center (MDACC). Eligible participants were 18 years or older at the time of enrollment and were either proven to be carriers or obligate carriers of a pathogenic or likely-pathogenic variant in one of the four MMR genes (*MLH1*, *MSH2*, *MSH6*, or *PMS2*) or *EPCAM*. Patients underwent colonoscopy/flexible sigmoidoscopy per standard of care indications. After patient consent, we retrospectively and prospectively collected individual patient data with manual review from the electronic medical records in a

REDCap database. Data on hospital visits, colonoscopy results, and past surgeries were collected for analysis. Loss to follow-up (LTFU) was defined as the absence of patient contact with clinic or procedure visits for more than 36 months at the latest captured point in data collection (censored in March of 2020).

Aims

The primary endpoints of this study were to (1): identify the degree of association between MMR genetic variation and the development of colorectal adenomas, advanced adenomas, and CRCs in our LS institutional cohort; and (2) determine the association of demographic characteristics with the development of colonoscopy findings during follow-up.

Statistical analysis

Patient demographics were summarized by descriptive statistics including surgical history (Table 1). The category of 'other surgeries' included the following procedures: subtotal colectomy with ileorectal anastomosis, abdominoperineal resection, low anterior resection, and partial colectomy. 'Small bowel surgeries' included small bowel resections with enterostomy and Billroth II gastrojejunostomies. Data on 'adenomas' as a whole included tubular adenomas, sessile serrated adenomas, and tubulovillous adenomas. Therefore, we did not incorporate hyperplastic or inflammatory polyps in outcome assessment. Advanced adenomas were defined as adenomas ≥ 1 centimeter in diameter, presenting villous features, and/or presence of high-grade dysplasia (HGD). Mean number adenomas per procedure (MAP) was calculated as the number of all adenomas from colonoscopies over the number of all colonoscopies performed. The mean number of adenomas per positive procedure (MPP+) was calculated as the number of all adenomas from colonoscopies over the number of all colonoscopies with adenomas found.

We conducted two separate analyses in this study. The first one evaluated LS carrier characteristics by MMR gene for the entirety of our study period (2013-2020 and inclusive of a retrospective review of the data prior to 2013 in a fraction of the participants). Carrier characteristics were summarized by MMR gene (Table 2), and significance of these MMR variant characteristics was determined using Pearson's chi-squared (χ^2) test for categorical variables and analysis of variance (ANOVA) for continuous variables. Furthermore, multivariate analyses were performed for adenomas, advanced adenomas, and CRC. These analyses were controlled for MMR gene variation, age, gender, ethnicity, race, smoking status, surgical history, and cancer status (defined as previvor compared to active cancer and survivor) over the entire study period. The results were reported as odds ratios (ORs) using logistic regressions. In the second one, we evaluated the time to our outcome(s) of interest for each patient for a 36-month period, which was the minimum period of follow-up for all participants in our cohort, using Kaplan-Meier cumulative incidence curves. These curves provided a descriptive overview of the time to incidence of adenoma, advanced adenoma, and CRC during the 36-month follow-up. We determined these

TABLE 1 Patient demographics.

Demographics	(N=163)
Age at Enrollment	
18-29	20 (12.3%)
30-39	29 (17.8%)
40-49	31 (19.0%)
50-59	42 (25.8%)
60-69	29 (17.8%)
70-79	10 (6.1%)
>80	1 (0.6%)
Sex	
Female	95 (58.3%)
Male	68 (41.7%)
Race	
White or Caucasian	128 (78.5%)
Black or African American	6 (3.7%)
Asian	7 (4.3%)
American Indian	1 (0.6%)
Unknown Race/Ethnicity	1 (0.6%)
Other	20 (12.3%)
Ethnicity	
Hispanic or Latino	22 (13.5%)
Not Hispanic or Latino	134 (82.2%)
Unknown	7 (4.3%)
Cancer History (n)*	113 (69.3%)
Colon	68 (60.2%)
Rectum	14 (12.4%)
Small Bowel	1 (0.9%)
Urothelial Tract	5 (4.4%)
Endometrial	23 (20.4%)
Other	43 (38.1%)
Genes	
MLH1	54 (33.1%)
MSH2/EPCAM/TACSTD1	60 (36.8%)
MSH6	33 (20.2%)
PMS2	16 (9.8%)
Colorectal surgery	71 (43.6%)
Right Hemicolectomy	36 (50.7%)
Total Colectomy with Ileostomy	1 (1.4%)
Total Colectomy with Ileorectal Anastomosis	4 (5.6%)
Left Hemicolectomy	6 (8.5%)

(Continued)

TABLE 1 Continued

Demographics	(N=163)
Sigmoid Colon Resection	5 (7.0%)
Other Surgeries	16 (22.5%)
Small Bowel Resection	3 (4.2%)

*, Note that patients can present with more than one cancer type.

incidences by MMR gene, ethnicity, and gender. Carriers were considered at-risk from the point they entered our cohort until they developed the outcome of interest (i.e., adenoma, advanced adenoma, or CRC). At the time of outcome development, patients were no longer considered at-risk and were not included in the analysis for the subsequent months. As some patients were found to have developed their first adenoma, advanced adenoma, or CRC at the time of enrollment, they were not included within the Kaplan-Meier cumulative incidence analysis. Death was included as a competing risk in our analysis, though no patients died prior to developing the outcomes of interest. For Kaplan-Meier cumulative incidence curves, statistical significance was determined using a Cox proportional regression analysis with Breslow method, and the results were reported as hazard ratios (HRs). For all analyses, a *P*-value≤0.05 was considered statistically significant. All data were analyzed using STATA v16.0 (STATA Corp., TX, US).

Results

Demographic characteristics of study participants

A total of 163 patients were enrolled in our cohort from 2013 to 2020, with visits and patient data spanning from 1997 to 2020 (Table 1): 33.1% carried a variant in *MLH1*, 36.8% in *MSH2/EPCAM*, 20.2% in *MSH6*, and 9.8% in *PMS2*. Overall, 58.3% were female, 79% were White, 4% Black, 4% Asian, 1% American Indian, and 12% were classified as other race. Regarding ethnicity, 14% identified themselves as Hispanic/Latino. 69% of patients in this cohort had previous history of cancer (survivors), with most survivors having CRC. Of all participants in the study, 29% reported a prior history of smoking and 12% were current smokers. Furthermore, 21% of participants self-reported that they were taking aspirin. Finally, a total of 7% of participants met criteria to be considered LTFU at the time of data analysis. Surgical history was also broken down by MMR gene (Supplementary Table 1) and described by survivorship status with most surgeries being right hemicolectomy procedures (Supplementary Table 2). We found that rates of right hemicolectomy were significantly higher among MLH1 and MSH2/EPCAM carriers and that survivors and patients with active cancer had a higher prevalence of colorectal surgeries, as expected (both, $P<0.001$).

Outcomes

From a total of 761 clinic visits, 596 were colonoscopies while the remaining 165 visits included upper GI procedures and non-procedural visits. Among the colonoscopies, 25.3% were performed before 2013, and 74.7% from 2013 onwards. Patients had an average of 10 colonoscopies (IQR, 6-14; range, 2-24, Table 2), with an average age at first colonoscopy of 46 years (IQR, 37-55; range, 18-73) and an average age at subsequent follow-up of 53 years (IQR, 44-62; range, 18-82). At the first colonoscopy, 21.4% of the patients had at least one adenoma. Of these patients, 66.7% had tubular adenomas, 15.2% sessile serrated adenomas, and 18.2% advanced adenomas.

TABLE 2 LS Carrier Characteristics by MMR Gene.

	MLH1	MSH2/EPCAM	MSH6	PMS2	Total	P-Value
N	54 (33.1%)	60 (36.8%)	33 (20.2%)	16 (9.8%)	163	
Status at Enrollment						0.154
Previvor	19 (35.9%)	22 (37.3%)	13 (40.6%)	6 (37.5%)	60 (37.0%)	
Survivor	18 (34.0%)	23 (39.0%)	10 (31.3%)	9 (56.3%)	61 (37.7%)	
Active Cancer	16 (30.2%)	14 (23.7%)	9 (28.13)	1 (6.3%)	41 (25.3%)	
Age at enrollment	42.6 (IQR, 34-50; range, 18-73)	44.5 (IQR, 33-53; range, 18-77)	51.9 (IQR, 44-61; range, 20-71)	46.5 (IQR, 33-59; range, 24-72)	46.0 (IQR, 35-56; range, 18-77)	0.082
Mean Interval Between Follow-Up (Months)	12.5 (IQR, 0-42; range, 0-128)	14.2 (IQR, 0-50; range, 0-118)	12.5 (IQR, 0-35; range, 0-59)	10.5 (IQR, 2-13; range, 1-31)	13.1 (IQR, 0-59; range, 0-128)	0.188
Mean Total Follow-Up Period (Years)	9.2 (IQR, 0.4-22.0; range, 0-22.0)	9.3 (IQR, 0.3-21.3; range, 0-21.3)	7.3 (IQR, 1.2-14.4; range, 0.6-14.4)	4.4 (IQR, 1.1-8.8; range, 0.1-8.8)	8.7 (IQR, 0-22.0; range, 0-22.0)	<0.001
Sex						0.336
Female	23 (43.4%)	37 (61.7%)	21 (63.6%)	11 (68.8%)	95 (58.3%)	

(Continued)

10.3389/fonc.2023.1146825

TABLE 2 Continued

	MLH1	MSH2/EPCAM	MSH6	PMS2	Total	P-Value
Male	30 (56.6%)	23 (38.3%)	12 (36.4%)	5 (31.3%)	68 (41.7%)	
Race						0.555
White or Caucasian	37 (69.8%)	47 (79.7%)	28 (87.5%)	13 (81.3%)	127 (78.4%)	
Black or African American	2 (3.8%)	1 (1.7%)	2 (6.3%)	1 (6.3%)	6 (3.7%)	
Asian	5 (9.4%)	1 (1.7%)	1 (3.1%)	0 (0.0%)	7 (4.3%)	
American Indian	1 (1.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.6%)	
Unknown Race	0 (0%)	9 (15.3%)	0 (0.0%)	0 (0.0%)	20 (12.4%)	
Other	8 (15.1%)	1 (1.7%)	1 (3.1%)	2 (12.5%)	1 (0.6%)	
Ethnicity						0.479
Hispanic	10 (18.5%)	9 (15.3%)	1 (3.1%)	2 (12.5%)	22 (13.5%)	
Non-Hispanic	41 (75.9%)	48 (81.4%)	29 (90.6%)	14 (87.5%)	134 (82.2%)	
Unknown	3 (5.6%)	2 (3.4%)	2 (6.3%)	0 (0%)	7 (4.3%)	
First Colonoscopy						
Mean Age (years)	43.7 (IQR, 36-50; range, 25-73)	43.8 (IQR, 36-52; range, 18-72)	51.4 (IQR, 42-61; range, 20-71)	49.5 (IQR, 32-59; range, 24-72)	45.8 (IQR, 37-55; range, 18-73)	0.043
Adenoma Count						0.959
0	21 (58.3%)	19 (48.7%)	8 (44.4%)	6 (66.7%)	54 (53.5%)	
1	6 (16.7%)	13 (33.3%)	6 (33.3%)	2 (22.2%)	27 (26.7%)	
2	3 (8.3%)	1 (2.6%)	2 (11.1%)	1 (11.1%)	7 (6.9%)	
≥3	1 (2.8%)	2 (5.1%)	1 (5.6%)	0 (0.0%)	4 4.0%)	
Advanced Adenoma Count	2 (5.6%)	2 (5.1%)	1 (5.6%)	0 (0.0%)	4 (4.0%)	0.479
CRC Count	3 (8.3%)	2 (5.1%)	0 (0.0%)	0 (0.0%)	5 (5.0%)	0.667
Screening and Colonoscopy Results						
Number of Colonoscopies	184 (30.9%)	271 (45.5%)	107 (18.0%)	29 (4.9%)	596	<0.001
Mean Number of Colonoscopies Per Patient	10.9 (IQR, 6-17; range, 2-24)	10.5 (IQR, 6-18; range, 2-22)	8.0 (IQR, 5-10; range, 2-15)	6.3 (IQR, 3-11; range, 2-11)	9.9 (IQR, 5-14; range, 2-24)	<0.001
Mean Number of Adenomas Per Colonoscopy Procedure (MAP)	0.32 (IQR, 0-3; range, 0-4)	0.61 (IQR, 0-5; range, 0-7)	0.82 (IQR, 0-5; range, 0-5)	0.44 (IQR, 0-1; range, 0-2)	0.54 (IQR, 0-5; range, 0-7)	<0.001
Mean Number of Adenomas Per Positive Colonoscopy Procedure (MPP+)	1.39 (IQR, 1-3; range, 1-4)	1.59 (IQR, 1-5; range, 1-7)	1.66 (IQR, 1-5; range, 1-5)	1.33 (IQR, 1-1; range, 1-2)	1.55 (IQR, 1-5; range, 1-7)	0.637
Mean Number of Advanced Adenomas Per Colonoscopy Procedure	0.01 (IQR, 0-0; range, 0-2)	0.03 (IQR, 0-1; range, 0-2)	0.04 (IQR, 0-1; range, 0-2)	0.02 (IQR, 0-0; range, 0-1)	0.02 (IQR, 0-2; range, 0-2)	0.391
Mean Number of Adenomas Per Patient For Follow-Up Year	0.31 (IQR, 0-1.86; range, 0-3.88)	0.90 (IQR, 0-4.08; range, 0-4.08)	0.84 (IQR, 0-2.50; range, 0-4.34)	0.56 (IQR, 0-0.91; range, 0-1.87)	0.66 (IQR, 0-4.08; range, 0-4.34)	<0.001
Mean Number of Advanced Adenomas Per Patient For Follow-Up Year	0.03 (IQR, 0-1.90; range, 0-1.90)	0.05 (IQR, 0-0.92; range, 0-0.92)	0.07 (IQR, 0-0.80; range, 0-0.80)	0.03 (IQR, 0-0.11; range, 0-0.11)	0.04 (IQR, 0-1.90; range, 0-1.90)	0.089
CRC Count	12 (22.2%)	5 (8.3%)	2 (6.1%)	0 (0%)	19 (11.7%)	0.002

Note that patients can present with more than one adenoma or advanced adenoma.

The MAP was calculated as 0.54 (IQR, 0-5; range, 0-7, Table 2). We observed statistically significant differences in the number of adenomas among the different MMR gene carriers (P<0.001) with *MSH6* carriers having the most adenomas (0.82; IQR, 0-5; range, 0-5) followed by *MSH2/EPCAM* (0.61; IQR, 0-5; range, 0-7), *PMS2* (0.44; IQR, 0-1; range, 0-2) and *MLH1* (0.32; IQR, 0-3; range, 0-4). The MPP+ was calculated as 1.55 (IQR, 1-5; range, 1-7) with no statistically significant difference between MMR carriers (P=0.637). The mean number of adenomas per patient for each year of follow-up was 0.66 (IQR, 0-4.08; range, 0-4.34) with *MSH2/EPCAM*

carriers having the greatest average number of adenomas per year relative to other carriers (IQR, 0-4.08; range, 0-4.08; $P<0.001$). The mean number of advanced adenomas per procedure was 0.02 (IQR, 0-2; range, 0-2), though we found no statistically significant difference by MMR pathogenic variant carriers ($P=0.391$). The mean number of advanced adenomas per patient for each year of follow-up was 0.04 (IQR, 0-1.90; range, 0-1.90) with no statistically significant difference between MMR variant carriers ($P=0.089$). The mean interval between follow-up was 13.1 months (IQR, 0-59; range, 0-128), and the mean duration of follow-up in our cohort over the entire enrollment period was 8.7 years (IQR, 0-22, range, 0-22). The mean interval between colonoscopies was 13.9 months (IQR, 11-15; range, 0-128) and this was not significantly different among the MMR gene groups, thus reflecting previous historical surveillance recommendations. Moreover, we found no statistically significant difference in the mean intervals between colonoscopies by race or ethnicity ($P=0.580$, $P=0.124$, respectively).

A total of 19 patients were diagnosed with CRCs within our cohort for the total follow-up period. Nine patients were referred to our institution with the diagnosed cancer, and five were diagnosed at their first colonoscopy. Of the remaining patients, three were diagnosed on their second visit and two on subsequent visits. Moreover, two of the 19 patients displayed metachronous tumors that occurred within a year of their first tumor diagnosis. A total of three of 19 patients had *in-situ* (stage 0), four stage I, six stage II, and five stage III tumors. There was missing stage information for one patient. Twelve of these tumors (22.2%) were diagnosed in *MLH1* carriers, five (8.3%) from *MSH2/EPCAM*, and 2 (6.1%) from *MSH6* carriers (Table 2). For carriers who were not diagnosed with CRC in their initial visit, the average interval from last colonoscopy to diagnosis of CRC was 11.6 months (SD 6.5).

Multivariate analyses

We conducted multivariate regression analyses to investigate the association between LS carrier profiles with likelihood of developing adenomas and CRC, controlling for ethnicity, race, gender, age, smoking status, and surgical history for the entire enrollment period. Compared to *MLH1* carriers, we found no statistically significant difference in the likelihood of developing adenomas between *MSH2/EPCAM, MSH6*, and *PMS2* (Table 3). As expected, participants ≥45 years old were more likely to develop adenomas compared to younger participants (OR 2.61, 95% CI 1.08-1.84, $P=0.034$). We found no statistically significant difference between different racial groups and the likelihood of developing adenomas. Furthermore, we found that, compared to previvors, participants with a prior history of cancer or active CRC were less likely to develop adenomas, even when controlling for age, gender, ethnicity, race, gene variant, and surgical history (OR 0.32, 95% CI 0.13-0.80, $P=0.015$). Surgical history was not associated with a statistically significant difference in the likelihood of adenoma development ($P=0.868$).

We assessed the association between gene variation and likelihood of developing an advanced adenoma (Table 4) and CRC development (Table 5) in which we controlled for the same covariates as in our prior analysis. While we controlled for both race and ethnicity in measuring the association between gene variant and advanced adenomas, this regression was only adjusted by race as, when controlling for race and ethnicity, the logistic regression did not converge. However, we did not find any statistically significant association between any of our measured variables and the likelihood of developing an advanced adenoma or CRC, likely due to the relatively sparse number of advanced adenomas and CRC cases within our cohort.

Cumulative incidence analysis

To assess the incidences for adenomas, advanced adenomas, and CRC within the first 36 months following enrollment, we conducted Kaplan-Meier cumulative incidence analyses and tested for significance using Cox proportional hazard regression. Figure 1A presents the cumulative incidence of all adenomas by MMR gene variation for 36 months. Carriers were considered at-risk from time of enrollment to first adenoma development if they did not have an adenoma at their initial visit. We conducted Cox proportional hazard regression for the risk of developing adenomas by MMR gene carriers. Compared to *MLH1* carriers, we found that *MSH2/EPCAM* carriers were more likely to develop adenomas (HR 2.17, 95% CI 1.01-4.66, $P=0.047$). Figure 1B presents the cumulative incidence of all adenomas by ethnicity in the same period. Overall, there is a non-statistically significant trend for Hispanics having a higher cumulative incidence of all adenomas and for developing adenomas earlier when compared to non-Hispanics patients ($P=0.073$). Figure 1C represents the cumulative incidence of all adenomas by gender displaying no significant differences in the incidence of adenomas when compared males to females in our cohort ($P=0.473$). Figure 1D represents the cumulative incidence of all adenomas stratified by age at enrollment. Patients 45 years or older had a significantly higher incidence of adenoma development than patients under 45 (HR 2.99, 95% CI 1.38-6.51, $P=0.006$). Regarding advanced adenomas, Figure 2A presents the cumulative incidence by MMR gene during the period of follow-up. Patients were considered at-risk from time of enrollment to the development of first advanced adenoma captured under surveillance if they did not have an advanced adenoma or CRC diagnosed at their initial visit. The Kaplan-Meier curve demonstrates a pattern where *PMS2* carriers appeared to have the greatest cumulative incidence of advanced adenomas in our cohort while the *MSH2/EPCAM* carriers had an earlier cumulative incidence for these adenomas. Importantly, however, we found no statistically significant differences in the incidence of advanced adenoma between *MLH1* and *MSH2/EPCAM, MSH6*, and *PMS2* ($P=0.105$, $P=0.111$, $P=0.093$, respectively). Figure 2B represents the cumulative incidence of CRC by genetic variant. Patients were considered at-risk for CRC from study enrollment to CRC development if they were not diagnosed with CRC at their initial visit. We did not observe differences between *MLH1* and other carrier subgroups ($P=0.198$). Finally, Figure 3 represents the cumulative incidence of colorectal adenoma

10.3389/fonc.2023.1146825

TABLE 3 Multivariate logistic regression for likelihood of adenoma development.

Factor	OR	95% CI	*P*-Value
MMR Variant			
MLH1	1	–	–
MSH2/EPCAM	0.97	0.50-1.89	0.923
MSH6	1.70	0.78-3.70	0.18
PMS2	1.16	0.32-4.20	0.816
Race			
White or Caucasian	1	–	–
Black or African American	–	–	–
Asian	0.49	0.06-4.30	0.519
American Indian	–	–	–
Other	–	–	–
Unknown	0.8	0.19-1.97	0.636
Ethnicity			
Non-Hispanic	1	–	–
Hispanic	1.47	0.91-2.39	0.118
Sex			
Male	1	–	–
Female	1.02	0.57-1.84	0.937
Age			
Age <45	1	–	–
Age ≥45	2.61	1.08-1.84	0.034
Smoking Status			
No documented smoking history	1	–	–
Prior or current smoker	0.8	0.52-1.23	0.304
Status			
Previvor	1	–	–
Survivor and Active Cancer	0.32	0.13-0.80	0.015
Surgical History			
No Surgical History	1	–	–
Surgical History	0.95	0.51-1.75	0.868

OR, Odds Ratio; CI, Confidence Intervals.

progression to CRC in our cohort for this same period. We did not observe an association between adenoma development and CRCs (*P*=0.160).

Discussion

Despite the well-known relationship between the MMR variants and CRC risk that has led to the implementation of intense screening programs through colonoscopy, LS carriers continue to be diagnosed with CRC. In this study, we analyzed a demographically diverse cohort of LS patients from a single institution tertiary care center engaged in LS screening through several decades and reported associations between carrier characteristics with outcome development. We performed logistic regression analyses to assess the likelihood of these outcomes for MMR gene, race and ethnicity, sex, age, smoking status, surgical history, and cancer status across all collected patient data.

We also performed cumulative incidence analysis of adenomas and advanced neoplasia (i.e., advanced adenomas and CRCs) for these carriers during a 36-month follow-up period. To visually represent the cumulative incidence for adenomas and advanced

10.3389/fonc.2023.1146825

TABLE 4 Multivariate logistic regression for likelihood of advanced adenoma development OR, Odds Ratio; CI, Confidence Intervals.

Factor	OR	95% CI	*P*-Value
MMR Variant			
MLH1	1	–	–
MSH2/EPCAM	0.72	0.16-3.16	0.659
MSH6	0.84	0.13-5.41	0.852
PMS2	1.77	0.16-19.43	0.639
Race			
White or Caucasian	1	–	–
Black or African American	–	–	–
Asian	–	–	–
American Indian	–	–	–
Unknown Race/Ethnicity	–	–	–
Other	1.02	0.155-6.79	0.985
Ethnicity			
Non-Hispanic Ethnicity	1	–	–
Hispanic Ethnicity	1.56	0.57-4.28	0.384
Sex			
Male	1	–	–
Female	0.96	0.25-3.74	0.951
Age			
Age <45	1	–	–
Age ≥45	0.78	0.12-4.90	0.788
Smoking Status			
No documented smoking history	1	–	–
Prior or current smoker	1.76	0.52-6.01	0.366
Status			
Previvor	1	–	–
Survivor and Active Cancer	0.52	0.08-3.57	0.505
Surgical History			
No Surgical History	1	–	–
Surgical History	1.04	0.24-4.47	0.961

neoplasia during this period, we generated Kaplan-Meier curves and tested their significance using Cox proportional regression.

Current guidelines by the National Comprehensive Cancer Network (NCCN) suggest earlier and more frequent colonoscopies in patients with *MLH1, MSH2*, and *EPCAM* variants. However, there is not yet a consensus on interval screening for this patient population. The British Society of Gastroenterology/Association of Coloproctology of Great Britain and Ireland (BSG/ACPBGI) guidelines has recommended interval screenings every 2 years for all patients with known MMR variants (12). In contrast, the American College of Gastroenterology's guidelines recommend yearly screenings in patients with known MMR variants but every 2 years for those who might be at risk or affected by LS (13). Here, we report a statistically significant greater and an earlier incidence of adenomas for *MSH2*/*EPCAM* carriers compared to *MLH1* over a 36-month follow-up period. However, we did not observe statistically significant associations between adenomas and MMR carriers. These findings support the NCCN's recommendations to start screening colonoscopies earlier in *MSH2* carriers, as they may be more likely to develop adenomas earlier compared to other LS carriers. However, the NCCN's guidelines propose time wide screening intervals (i.e., 1-3 years) rather than

10.3389/fonc.2023.1146825

TABLE 5 Multivariate logistic regression for likelihood of CRC.

Factor	OR	95% CI	*P*-Value
MMR Variant			
MLH1	1	–	–
MSH2/EPCAM	0.74	0.11-5.23	0.764
MSH6	0.84	0.07-9.46	0.888
PMS2	–	–	–
Race			
White or Caucasian	1	–	–
Black or African American	–	–	–
Asian	4.39	0.28-75.5	0.287
American Indian	–	–	–
Other	1.41	0.15-15.52	0.728
Unknown	–	–	–
Sex			
Male	1	–	–
Female	0.28	0.03-2.95	0.288
Age			
Age <45	1	–	–
Age ≥45	0.67	0.08-5.53	0.709
Smoking Status			
No documented smoking history	1	–	–
Prior or current smoker	0.81	0.25-2.62	0.728
Status			
Previvor	1	–	–
Survivor and Active Cancer	–	–	–
Surgical History			
No Surgical History	1	–	–
Surgical History	1.2	0.20-7.31	0.842

OR, Odds Ratio; CI, Confidence Intervals.

individualized guidance, which permits significant variability in colonoscopy frequency by individual providers and centers' preferences. Analysis from the Prospective Lynch Syndrome Database (PLSD) showed that colonoscopies more frequent than once every three years did not necessarily reduce the incidence of CRC or outcomes upon diagnosis (4). In addition, analysis from this database showed that the removal of adenomas in a LS patient cohort did not decrease the incidence of CRC. In practical terms, these intervals are often adjusted to reflect the individual occurrence of adenomas in each LS carrier. Our adenoma incidence findings of *MSH2/EPCAM* carriers are in line with others previously documented in the literature; however, as stated, we did not observe a statistically significant difference in prevalence of adenoma between MMR variants on multivariate analysis when the entire follow-up for the cohort was considered. This discrepancy may be attributable to size of the cohort, the increased surveillance for the participants in our study relative to the general LS population and the influence of surgical history among survivors. As our Kaplan-Meier curves measured incidence for the first 36-months following the initial appointment, this discrepancy may also suggest that the likelihood of adenoma development for *MSH2* and *EPCAM* may be attenuated following consistent surveillance. These suggestive findings provide additional information on pre-cancer incidence that can help to tailor screening intervals by MMR genotype. Based on expert opinion, LS patients who benefit from annual rather than biennial or triennial coloscopies are those with a prior history of CRC or adenomas, carriers greater than 40 years of age and males (11). Although our findings show that male carriers have a higher incidence of adenomas, this was not determined to be statistically significant on Cox proportional regression or

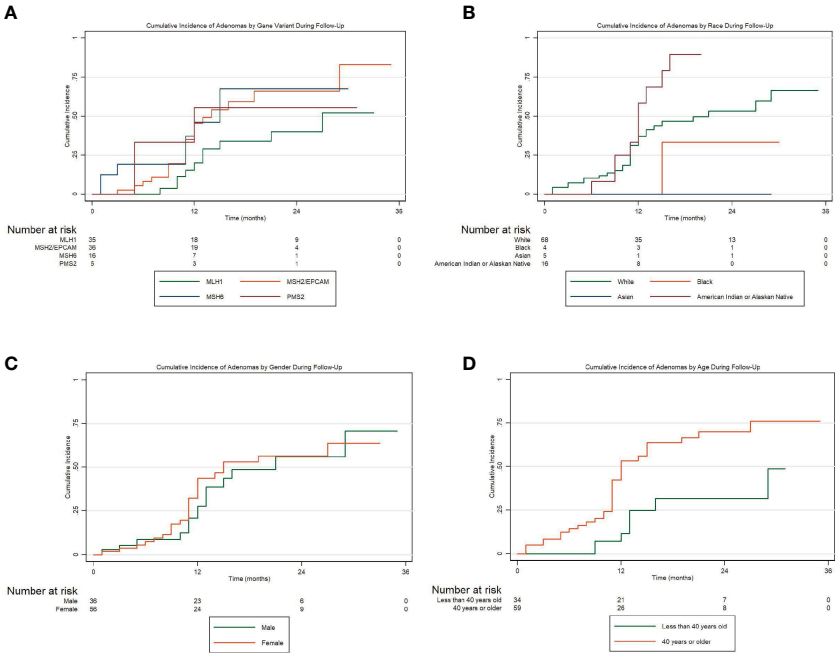


FIGURE 1
(A) Cumulative incidence of all colorectal adenomas by MMR gene variation over a period of 36 months of follow-up; **(B)** Cumulative incidence of all adenomas stratified Hispanic ethnicity; **(C)** Cumulative incidence of all adenomas stratified by gender; **(D)** Cumulative incidence of all adenomas stratified by age.

multivariate analysis. Our findings demonstrated a significantly increased likelihood of adenoma development within our cohort for participants 45 years or older. Further, we found that participants with an active or prior history of cancer were less likely to develop subsequent adenomas on multivariate analysis. While we found a statistically significant difference in the rate of prior colorectal surgeries by MMR gene carrier, surgical history was not associated with adenoma development on multivariate analysis. Therefore, more studies are needed to validate these recommendations and to find more data-driven associations that can improve the level of evidence behind current guidelines to match the clinical reality.

Based on our results, we propose that other patient demographics, specifically race and ethnicity, should be considered in estimating the risk of developing CRC for LS patients. Although LS carriers carry significant lifetime risk for the development of CRC regardless of ethnicity and race, patients belonging to racial and ethnic minorities in the United States are less likely to receive genetic evaluation for inherited CRC syndromes and may not receive sufficient screening following diagnosis (15). This discrepancy has been correlated to a lack of patient education on adequate screening modalities leading to stigmatization of screening practice, the potential effects of oncologists' implicit biases in patient-physician interactions, and the insurance disparities between Hispanic and non-Hispanic populations (16–18). In contrast to the literature, we showed no significant difference in interval screening between Hispanics and non-Hispanics in our cohort. This observation may be attributable to the consistency in screening practices within our cohort referred to a tertiary care center or may otherwise indicate that LS carriers enrolled in our study may be less likely to face those barriers to care. Furthermore, while Hispanic carriers had a greater and earlier incidence of adenomas compared to non-Hispanic patients, this difference was also not determined to be significant on Cox proportional hazard or multivariate analysis. To our knowledge, this is the first analysis that specifically controlled for ethnicity when evaluating cumulative adenoma incidence over a follow-up period in a LS population. While prior investigations have shown an increased mortality rate within CRC for these populations, to our knowledge, no study has specifically investigated the time to adenoma development for this patient cohort (19). Given that Hispanic patients often develop adenomas and CRC at a younger age, have a greater incidence of *MLH1* and *PMS2* variants, and are less likely to receive appropriate screening, these patients represent a vulnerable population that may require further analysis for evidence-based screening and management guidelines (14). Therefore, our findings highlight the need for further investigation into potential disparities in screening and effective interventions for Hispanic LS carriers.

We acknowledge several limitations of our study. While the enrolled patient cohort was demographically diverse, we recognize that the population analyzed may have limited generalizability to LS patients worldwide. Second, we did not establish a significant difference in patient outcomes based on MMR profiles or ethnicity. Because patients enrolled in our study underwent regular screenings, the outcomes for the LS patient cohort may

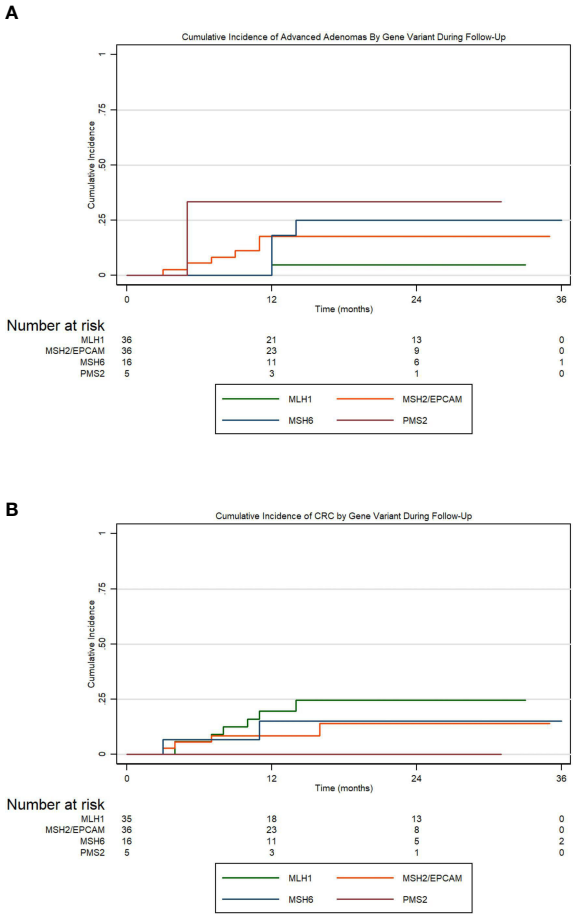


FIGURE 2
(A) Cumulative incidence of advanced adenomas by MMR variation over a period of 36 months of follow-up; **(B)** Cumulative incidence of colorectal cancer by MMR gene variation.

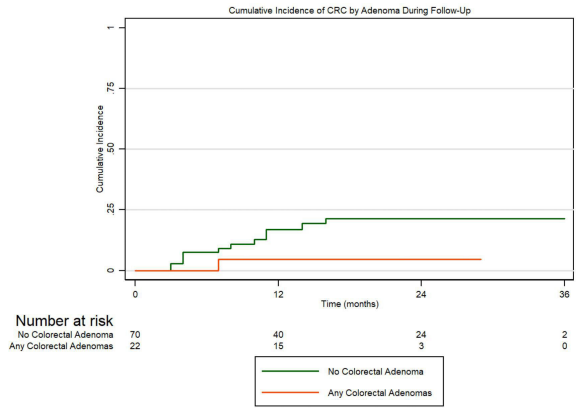


FIGURE 3
Cumulative incidence of colorectal adenoma progression to CRC.

not reflect the outcomes in an unmonitored patient population. Third, our follow-up period was limited to 36 months post-enrollment, so we did not generate differences in follow-up among participants that would have been difficult for us to control. We continue to follow up the outcomes in our patient cohort, but the generalizability of our current results reported here is limited within this 36-month period. Therefore, longer monitoring may reveal more robust and generalizable clinical outcomes. Fourth, we did not collect specific information on the prevalence of other LS-related cancers; thus, we did not capture the risk for gynecological or other GI tumors. Fifth, we were unable to account for the potential influence of the significant advancements in endoscopic technologies from the earliest available patient data (in 1997) to present and did not capture Key Performance Indicators (KPI) to approximate for the quality of colonoscopies performed for this cohort. Sixth, we described the number of patients who reported taking aspirin, but we did not systematically collect data on aspirin usage such as dosing throughout the study and did not therefore account for this in our multivariate analysis. Finally, we did not exclude patients with a history of colorectal surgery, which limits the potential for adenoma and CRC development in a subgroup of LS survivors.

Our study demonstrates several strengths. The patient cohort represented patients from various age groups, which permits greater generalizability of our results to a larger patient cohort. Furthermore, we captured the frequency and onset of adenomas for a vulnerable patient population and further stratified by ethnicity. We were also able to follow the patient cohort over an extended period with minimal loss to follow-up, which allowed us to thoroughly monitor the incidence of adenoma development. As the database continues to be updated, the correlation between demographic information and potential outcomes of interest may improve. LS patients in our database were collected and analyzed for adenoma incidence based on demographic information (such as race and gender) and MMR carrier status. We further investigated the degree to which these characteristics were associated with advanced adenomas and the development of CRC. The maintenance of a robust database of LS patients with a variety of heredity MMR variants is necessary for the creation of biobanks with prospective tissue collection, providing greater insights into the carcinogenesis of LS from a multi-specialty perspective. This database will allow providers to more precisely tailor treatment plans to individual patients based on constitutional variant status and patient characteristics. Finally, while we observed that Hispanic carriers experienced a greater cumulative incidence of adenomas, we did not observe a statistically significant difference in the cumulative incidence of adenomas by Hispanic ethnicity for this cohort. These results highlight the need for a well-maintained database of LS patients to facilitate proper surveillance and appropriate intervention management for Hispanic LS carriers, as they are not necessarily more prone to the development of adenomas compared to non-Hispanic carriers. Therefore, given the deficiencies in the screening infrastructure documented extensively in the medical literature for Hispanic patients and racial minorities, further studies are warranted to determine the efficacy of established LS guidelines for demographically diverse patient populations and modify them accordingly.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by MD Anderson Cancer Center IRB. The patients/participants provided their written informed consent to participate in this study.

Author contributions

EV contributed to the conception and design of the study. PL, MR-B, ST, MT, YY, DW, VS, and EV were involved in patient enrollment. JM, MM, PL, MR-B, ST, MT, YY, and EV identified the study subjects and provided clinical information. LR-U, JK, KE, and CB organized and maintained the database. GDC and LR-U performed statistical analysis. GDC, LR-U, DG, and EV wrote the manuscript and provided revisions of the manuscript. EV provided critical review of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by grant R01 CA219463 (US National Institutes of Health/National Cancer Institute), and a gift from the Feinberg Family to EV, and P30 CA016672 (US National Institutes of Health/National Cancer Institute) to the University of Texas MD Anderson Cancer Center Core Support Grant, P50 CA221707 (US National Institutes of Health/National Cancer Institute) to The University of Texas MD Anderson Cancer Center GI SPORE Grant, the generous philanthropic contributions to The University of Texas MD Anderson Cancer Center Moon Shots Program, and a grant from the University of Texas MD Anderson Cancer Center Duncan Family Institute for Cancer Prevention and Risk Assessment.

Acknowledgments

We thank the patients and their families for their participation. The authors are grateful to Karen Colbert Maresso for critically reading the manuscript.

Conflict of interest

Dr. Vilar has a consulting or advisory role with Janssen Research and Development, Recursion Pharma, Guardant Health, and Tornado/Cambrian. He has received research support from Janssen Research and Development.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1146825/full#supplementary-material

References

1. Sinicrope FA. Lynch syndrome-associated colorectal cancer. *N Engl J Med* (2018) 379(8):764–73. doi: 10.1056/NEJMcp1714533

2. Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP. Milestones of lynch syndrome: 1895-2015. *Nat Rev Cancer.* (2015) 15(3):181–94. doi: 10.1038/nrc3878

3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA: A Cancer J Clin* (2022) 72(1):7–33. doi: 10.3322/caac.21708

4. Møller P, Seppälä T, Dowty JG, Haupt S, Dominguez-Valentin M, Sunde L, et al. Colorectal cancer incidences in lynch syndrome: a comparison of results from the prospective lynch syndrome database and the international mismatch repair consortium. *Hereditary Cancer Clin Practice.* (2022) 20(1):36. doi: 10.1186s13053-022-00241-1

5. Møller P, Seppälä T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Incidence of and survival after subsequent cancers in carriers of pathogenic MMR variants with previous cancer: a report from the prospective lynch syndrome database. *Gut* (2017) 66(9):1657–64. doi: 10.1136/gutjnl-2016-311403

6. Valentin M, Sampson J, Seppälä T, Ten Broeke S, Plazzer J-P, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the prospective lynch syndrome database. *Genet Med* (2019) 22 15–25. doi: 10.1038/s41436-019-0596-9

7. Cerretelli G, Ager A, Arends MJ, Frayling IM. Molecular pathology of lynch syndrome. *J Pathol* (2020) 250(5):518–31. doi: 10.1002/path.5422

8. Brenner H, Altenhofen L, Stock C, Hoffmeister M. Natural history of colorectal adenomas: birth cohort analysis among 3.6 million participants of screening colonoscopy. *Cancer Epidemiol Biomarkers Prev* (2013) 22(6):1043–51. doi: 10.1158/1055-9965.EPI-13-0162

9. Hossain MS, Karuniawati H, Jairoun AA, Urbi Z, Ooi J, John A, et al. Colorectal cancer: a review of carcinogenesis, global epidemiology, current challenges, risk factors, preventive and treatment strategies. *Cancers (Basel)* (2022) 14(7). doi: 10.3390/cancers14071732

10. Sekine S, Mori T, Ogawa R, Tanaka M, Yoshida H, Taniguchi H, et al. Mismatch repair deficiency commonly precedes adenoma formation in lynch syndrome-associated colorectal tumorigenesis. *Mod Pathol* (2017) 30(8):1144–51. doi: 10.1038/modpathol.2017.39

11. Gupta S, Weiss JM, Axell L, Burke CA, Chen LM, Chung DC, et al. NCCN guidelines® insights: Genetic/Familial high-risk assessment: colorectal, Version 2, 2022. *J Natl Compr Canc Netw* (2022). doi: 10.1164/jnccn.2021.0048

12. Monahan KJ, Bradshaw N, Dolwani S, Desouza B, Dunlop MG, East JE, et al. Guidelines for the management of hereditary colorectal cancer from the British society of gastroenterology (BSG)/Association of coloproctology of great Britain and Ireland (ACPGBI)/United kingdom cancer genetics group (UKCGG). *Gut* (2020) 69(3):411–44. doi: 10.1136/gutjnl-2019-319915

13. Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW. ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol* (2015) 110(2):223–62. doi: 10.1038/ajg.2014.435

14. Muller C, Lee SM, Barge W, Siddique SM, Berera S, Wideroff G, et al. Low referral rate for genetic testing in racially and ethnically diverse patients despite universal colorectal cancer screening. *Clin Gastroenterol Hepatol* (2018) 16(12):1911–8.e2. doi: 10.1016/j.cgh.2018.08.038

15. Dharwadkar P, Greenan G, Stoffel EM, Burstein E, Pirzadeh-Miller S, Lahiri S, et al. Racial and ethnic disparities in germline genetic testing of patients with young-onset colorectal cancer. *Clin Gastroenterol Hepatol* (2022) 20(2):353–61.e3. doi: 10.1016/j.cgh.2020.12.025

16. Hann KEJ, Freeman M, Fraser L, Waller J, Sanderson SC, Rahman B, et al. Awareness, knowledge, perceptions, and attitudes towards genetic testing for cancer risk among ethnic minority groups: a systematic review. *BMC Public Health* (2017) 17 (1):503. doi: 10.1186/s12889-017-4375-8

17. Penner LA, Dovidio JF, Gonzalez R, Albrecht TL, Chapman R, Foster T, et al. The effects of oncologist implicit racial bias in racially discordant oncology interactions. *J Clin Oncol* (2016) 34(24):2874–80. doi: 10.1200/JCO.2015.66.3658

18. Ou JY, Warner EL, Nam GE, Martel L, Carbajal-Salisbury S, Fuentes V, et al. Colorectal cancer knowledge and screening adherence among low-income Hispanic employees. *Health Educ Res* (2019) 34(4):400–14. doi: 10.1093/her/cyz013

19. Carethers JM. Racial and ethnic disparities in colorectal cancer incidence and mortality. *Adv Cancer Res* (2021) 151:197–229. doi: 10.1016/bs.acr.2021.02.007

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 01 May 2023
DOI 10.3389/fonc.2023.1166238

Check for updates

OPEN ACCESS

EDITED BY
Christina Therkildsen,
Copenhagen University Hospital, Denmark

REVIEWED BY
Wei Chen,
The Ohio State University, United States
Kevin Monahan,
Imperial College London, United Kingdom

*CORRESPONDENCE
Andreas V. Hadjinicolaou
ah499@cam.ac.uk

†These authors have contributed equally to this work and share first authorship

RECEIVED 15 February 2023
ACCEPTED 11 April 2023
PUBLISHED 01 May 2023

CITATION
Williams MH, Hadjinicolaou AV, Norton BC, Kader R and Lovat LB (2023) Lynch syndrome: from detection to treatment. *Front. Oncol.* 13:1166238. doi: 10.3389/fonc.2023.1166238

COPYRIGHT
© 2023 Williams, Hadjinicolaou, Norton, Kader and Lovat. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Lynch syndrome: from detection to treatment

Madeleine H. Williams[1†], Andreas V. Hadjinicolaou[2,3*†], Benjamin C. Norton[4†], Rawen Kader[5] and Laurence B. Lovat[5]

[1]Department of Gastroenterology, Guy's and St. Thomas NHS Foundation Trust, London, United Kingdom, [2]Department of Gastroenterology, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom, [3]Early Cancer Institute, Department of Oncology, University of Cambridge, Cambridge, United Kingdom, [4]Department of Gastroenterology, University College London Hospitals NHS Foundation Trust, London, United Kingdom, [5]Wellcome-EPSRC Centre for Interventional and Surgical Sciences, University College London, London, United Kingdom

Lynch syndrome (LS) is an inherited cancer predisposition syndrome associated with high lifetime risk of developing tumours, most notably colorectal and endometrial. It arises in the context of pathogenic germline variants in one of the mismatch repair genes, that are necessary to maintain genomic stability. LS remains underdiagnosed in the population despite national recommendations for empirical testing in all new colorectal and endometrial cancer cases. There are now well-established colorectal cancer surveillance programmes, but the high rate of interval cancers identified, coupled with a paucity of high-quality evidence for extra-colonic cancer surveillance, means there is still much that can be achieved in diagnosis, risk-stratification and management. The widespread adoption of preventative pharmacological measures is on the horizon and there are exciting advances in the role of immunotherapy and anti-cancer vaccines for treatment of these highly immunogenic LS-associated tumours. In this review, we explore the current landscape and future perspectives for the identification, risk stratification and optimised management of LS with a focus on the gastrointestinal system. We highlight the current guidelines on diagnosis, surveillance, prevention and treatment and link molecular disease mechanisms to clinical practice recommendations.

KEYWORDS

lynch syndrome, mismatch repair (MMR) deficiency, colorectal cancer, surveillance, cancer diagnosis, cancer treatment

1 Introduction

Lynch Syndrome (LS) is a hereditary cancer predisposition syndrome characterised by a high lifetime risk of developing cancers, primarily colorectal and endometrial (1). These cancers exhibit microsatellite instability (MSI) due to defects in the cellular mismatch repair (MMR) system (2). LS is associated with other malignancies including gastrointestinal (GI) (e.g. gastric, small intestinal, hepato-biliary and pancreatic) and extra-GI cancers (e.g. prostate, ovaries, skin, central nervous system and upper urinary tract) (3). LS follows an autosomal dominant pattern of inheritance with germline

pathogenic variants in one of the MMR genes, which, in health, maintain genomic stability (4). An estimated 1/450 people in the UK have LS (5), and of those, only 5% are diagnosed. The lifetime risk of colorectal cancer (CRC) in LS patients can vary from 10-80% dependent on the MMR mutation and age, and it is thought to be responsible for 3-5% of all CRCs (6, 7). This makes LS one of the most frequently encountered cancer susceptibility syndromes.

A prototypical cancer surveillance programme using colonoscopy exists for CRC in the setting of LS, but quality data on the role of surveillance for other LS-associated tumours is limited. In recognition of the growing need for new approaches to improve survival, this review explores the current landscape and future perspectives for the detection, risk stratification and management of LS.

2 Identification

2.1 LS genetics

LS is due to a pathogenic variant within one of the MMR genes: *MLH1*, *MSH2*, *MSH6* or *PMS2* (4). *MLH1*/*MSH2* mutations are responsible for 70-90% of LS cases and carry significantly higher lifetime cancer risk (8). A small proportion of LS cases (1-3%) arise secondary to constitutional epimutations of the *MLH1* or *MSH2* genes (9). The heterozygous, loss-of-function, germline mutations in MMR genes are phenotypically dominant but may also convey vulnerability to a second, somatic mutation in the wildtype (normal) allele. Tumorigenesis then develops due to deficient mismatch repair (dMMR) and accumulation of further mutations including in small regions of repeated DNA called microsatellites. This gives rise to microsatellite instability (MSI); the genetic signature of LS-associated tumours.

The need to differentiate between sporadic and inherited CRC in patients with dMMR tumours is crucial because of downstream implications for cancer surveillance. Unfortunately, this is not always straightforward and we are increasingly aware of a heterogenous patient group with Lynch-like syndrome (LLS) defined as dMMR tumours where LS is suspected but no pathological germline MMR mutation is identified (10).

2.2 Diagnosis

The diagnosis of LS is made in symptomatic patients presenting with a LS-associated cancer, or among asymptomatic patients with a confirmed familial pathogenic variant. In symptomatic cases, the tumour is subjected to molecular profiling for evidence of dMMR. MSI is assessed either using polymerase chain reaction (PCR)-based testing or loss of/abnormal protein expression of *MLH1*, *MSH2*, *MSH6* or *PMS2* using immunohistochemistry (IHC) (11). Both methods have high sensitivity (PCR 92.9%, IHC 92.4%), specificity (PCR 86.3%, HCI 87.8%) and negative predictive values (PCR 99.6%, IHC 99.6%) for LS (12).

An abnormal result must be followed by referral for genetic testing and counselling. Younger patients (<40 years old) should be referred directly for germline testing according to the NHS National Genomic Medicine Service (GMS) Lynch Syndrome Project guidelines (13). Among families with a confirmed pathogenic MMR variant, asymptomatic patients can be referred for cascade genetic testing directly without the need for findings consistent with CRC.

Since 2017, the National Institute for Clinical Excellence (NICE) has recommended testing all newly identified CRCs for dMMR by IHC or for MSI to guide the need for LS evaluation (11). This guidance was expanded to IHC testing in all new endometrial cancers in 2020 (14). These recommendations have superseded the previously used Amsterdam Criteria and Bethesda Guidelines (15, 16) which mainly relied on crude measures such as family history and age of cancer onset (17). Looking to the future, NICE have proposed an accelerated review of next generation sequencing (NGS) as a potential index test for paired tumour-germline profiling in all newly diagnosed CRCs (18). NGS enables identification of MSI using computational algorithms such as mSINGS, MSISenory, and MANTIS among others (19). It can simultaneously sequence the whole exome looking for markers of MSI, compared to a normal/baseline sample, which is measured against a threshold value. Concurrently, exome tumour sequencing can be paired with a blood sample to enable differentiation between somatic and germline variants (20). This paired testing is superior to traditional stepwise testing, which would enable earlier, more precise and personalised risk stratification in suspected LS cases (21).

2.3 Determining cancer risk

Over the last few decades, there has been great insight into the natural history of LS patients with thousands of unique germline MMR gene variants identified and recorded in international databases such as InSiGHT (22). However, having a pathogenic variant does not result in a uniform diagnosis across all patients, with great genetic variability observed due to penetrance (i.e. the probability of a gene being expressed) and expressivity (i.e. if the gene is penetrant, the variability in that expression). The establishment of the Prospective Lynch Syndrome Database (PLSD), an international, multi-centre, observational prospective study, has improved understanding of the cumulative incidence and survival of LS-associated cancer patients (between 25-75 years) and equipped us with age and cancer-specific risk estimates for each pathogenic MMR variant (Table 1) (24, 25). However, it is important to acknowledge its limitations such as the absence of a control group who did not undergo surveillance and granular data such as cancer-specific survival.

These limitations have somewhat been addressed by the international multi-centre International Mismatch Repair Consortium (IMRC) (26). In contrast to the PLSD, in which all cases have undergone at least one colonscopy, IMRC data derives from retrospective segregation analysis of LS families, including older generations who did not receive comparable colonoscopic surveillance. Contrary to expectations, incidence of CRC in *path_MLH1* and *path_MSH2* carriers in the PLSD group (who

10.3389/fonc.2023.1166238

TABLE 1 Cumulative incidence of individual cancers in patients with pathogenic MMR variants between 25-75 years old (23).

Cancer type		Cumulative cancer risk at age 75 years (% (95% CI))			
		path_MLH1	*path_MSH2*	*path_MSH6*	*path_PMS2*
Colorectal	Colon	46.7 (39.2 to 54.3)	42.4 (32.9 to 51.9)	14.2 (3.1 to 25.4)	0
	Sigmoid and rectum	11.8 (7.2 to 16.4)	18.3 (10.9 to 25.6)	4.6 (0.0 to 9.7)	0
Gynaecological	Endometrium	42.7 (33.1 to 52.3)	56.7 (41.8 to 71.6)	46.2 (27.3 to 65.0)	26.4 (0.8 to 51.9)
	Ovaries	10.1 (4.8 to 15.4)	16.9 (5.7 to 28.0)	13.1 (0.0 to 31.2)	0
Upper GI	Stomach	7.1 (3.5 to 10.8)	7.7 (1.9 to 13.6)	5.3 (0.0 to 13.1)	0
	Duodenum	6.5 (2.7 to 10.2)	2.0 (0.1 to 4.0)	0	0
	Biliary	3.7 (1.3 to 6.2)	1.7 (0.0 to 5.1)	0	0
	Pancreas	6.2 (2.6 to 9.8)	0.5 (0.0 to 1.5)	1.4 (0.0 to 4.2)	0
Urinary tract	Bladder	4.1 (1.5 to 6.7)	8.1 (2.8 to 13.3)	8.2 (0.0 to 16.9)	0
	Kidneys and ureters	4.6 (1.6 to 7.6)	17.8 (10.6 to 25.0)	3.0 (0.0 to 7.0)	0
Other	Brain	1.0 (0.0 to 2.4)	5.3 (0.2 to 10.3)	1.4 (0.0 to 4.2)	0
	Prostate	16.9 (8.5 to 25.3)	31.6 (11.7 to 51.5)	18.3 (0.0 to 44.4)	37.9 (0.0 to 95.9)
	Breast	12.0 (6.7 to 17.3)	11.5 (4.6 to 18.4)	13.3 (2.2 to 24.4)	55.9 (0.0 to 100.0)

underwent colonoscopy and polypectomy) was significantly higher than in the IMRC series. Differences in data fidelity between the two databases could have influenced these findings (27).

3 Risk stratification

Over the last decade, significant improvements have been made in the personalised risk stratification of patients with LS. However, the optimal timing of surveillance is still to be determined and there is a paucity of data for extra-colonic tumours and surveillance in older age patients (28).

3.1 Colorectal cancer surveillance

Current consensus favours conoloscopy for CRC surveillance in asymptomatic patients with LS. A landmark prospective study from Finland in 2000 demonstrated that 3-yearly colonoscopy in LS decreased CRC incidence and mortality (29, 30), with other non-randomised studies replicating these findings (31, 32). However, many of these are somewhat limited in their granularity of data. For example, in the aforementioned study, all participants who attended a colonscopy were deemed to be compliant with surveillance regardless of the frequency of their surveillance or whether they had any actual further colonoscopies at all. More recently, a retrospective cohort study (33) used a unique time-based model to explore the effect of surveillance interval in LS (<27 months vs >27 months vs no surveillance), demonstrating that shorter intervals reduced the risk of first CRC diagnosis. These findings could encourage adherence to timely surveillance in at-risk individuals, although an important limation of this study in the context of colonoscopy, was the inclusion of other surveillance techniques such as CT colonography, MRI and barium enema.

The optimal strategy for CRC surveillance in LS remains the subject of ongoing research. Guidelines vary internationally (outlined in Table 2) (3, 10, 34–45), with the European Society of Gastrointestinal Endoscopy (ESGE) recommending 2 yearly (36). Interestingly, 98% of centres favoured colonscopy every 1-2 years when reported to the IMRC (46). The prevalence of CRC is low in patients with LS under the age of 25 regardless of genotype, however data from both PLSD and IMRC support the notion that those with the higher penetrance *MHL1* and *MSH2* variants typically develop CRC earlier in life than their *MSH6* and *PMS2* counterparts (26, 47), hence the decision by some to begin surveillance earlier for *MSH1/MSH2* carriers (Table 2). In patients with *PMS2* variants, carcinogenesis may be more akin to the traditional adenoma-carcinoma sequence (25, 48) leading to low CRC incidence which may justify the suggestion from the European Mallorca guidelines for 5-yearly surveillance (35).

Whilst 1-2 yearly colonoscopy in LS is widely practiced, prospective observational cohorts have demonstrated that lifetime risk of CRC, including metchronous tumours, remains as high as 36% (49, 50) and do not necessarily improve by increasing surveillance frequency (51). Analysis of 2747 LS patients showed no significant difference between incidence and stage of CRC between annual, 1-2 yearly and 3 yearly surveillance (52). It has also been suggested that frequent surveillance could lead to over-diagnosis by detecting tumours that may not have become clinically significant (53). Compliance issues too may be an argument for longer surveillance intervals. In one study, loss to follow-up rates were higher among participants randomised to annual screening than those having 2 or 5-yearly surveillance (54). Considering these findings it is perhaps unsurprising that consensus on surveillance strategy is difficult to establish.

There are various hypotheses as to why the rate of interval CRC is still high despite best efforts in surveillance programmes. First, it has been suggested that CRC in LS develops through accelerated tumorigenesis compared with sporadic CRC (55). This assumes a prior optimally performed colonoscopy. Second, adenomas in LS are often proximal, flat, and harder to detect, which could lead to missed lesions, especially during inadequately performed colonoscopy (56). Finally, LS-associated CRCs may have a unique, non-polypous carcinogenesis pathway that allow them to develop from endoscopically undectable lesions (e.g. colonic crypts) (57). The aforementioned failure to reduce CRC incidence by reducing surveillance intervals suggests that accelerated

TABLE 2 Current recommendations for colorectal cancer surveillance from different national and international organisations.

Country/ Continent of origin	Organisation	Age to start surveillance (with corresponding pathological MMR gene mutation, where applicable)	Surveillance interval (with corresponding MMR gene mutation, where applicable)	Comments
Australia	Cancer Institute of New South Wales (34)	25 years (*MLH1/MSH2)* 35 years (*MSH6/PMS2*)	1-2 years	Review all cases at age 60 years with a view to reducing frequency
Europe	Mallorca Guidelines from The European Hereditary Tumour Group (EHTG) and European Society of Coloproctology (ESCP) (35)	25 years (*MLH1/MSH2)* 35 years (*MSH6/PMS2*)	2-3 years (*MLH1/MSH2/ MSH6)* 5 years (*PSM2*)	
	European Society of Gastrointestinal Endoscopy (ESGE) Guideline (36)	25 years (*MLH1/MSH2)* 35 years (*MSH6/PMS2*)	2 years	
	European Society of Medicine (ESMO) (37)	25 years (*MLH1/MSH2)* 35 years (*MSH6/PMS2*)	1-2 years	Offer colonoscopy 5 years younger than age of youngest diagnosed CRC case in family (if diagnosed before age 25)
France	French National Authority for Health (38)	25 years	2 years	Offer colonoscopy 5 years younger than age of youngest diagnosed CRC case in family (if diagnosed before age 25)
Germany	German Consortium for Familial Colorectal Cancer (39)	25 years	1-2 years	
Japan	Japanese Society for Cancer of the Colon and Rectum (JSCCR) (40)	20-25 years	1-2 years	
Netherlands	Integrated Cancer Centre Netherlands (41)	25 years	2 years	
Spain	Spanish Society of Medical Oncology (SEOM) (42)	20-25 years	1-2 years	Offer colonoscopy 2-5 years younger than age of youngest diagnosed CRC case in family (if diagnosed before age 25)
UK	British Society of Gastroenterology BSG)/ Association of Coloproctology of Great Britain and Ireland (ACPGBI)/United Kingdom Cancer Genetics Group (UKCGG) (10)	25 years (*MLH1/MSH2*) 35 years (*MSH6/PMS2*)	2 years	Until age 75 years
USA	US Multi-Society Task Force (USMSTF) (43)	20-25 years *(MLH1/ MSH2)* 30 years (*MSH6*) 35 years (*PMS2*)	1 year	Offer colonoscopy 2-5 years younger than age of youngest diagnosed CRC case in family (if diagnosed before age 25)
	American College of Gastroenterology (ACG) (3)	20-25 years (*MLH1/ MSH2)* 25-30 years (*MSH6/ PMS2*)	1-2 years	
	American Society of Clinical Oncology (ASCO) (44) [1]	25 years (*MLH1/MSH2)* 35 years (*MSH6/PMS2*)	1-2 years	Offer colonoscopy 5 years younger than age of youngest diagnosed CRC case in family (if diagnosed before age 25)

(Continued)

TABLE 2 Continued

Country/ Continent of origin	Organisation	Age to start surveillance (with corresponding pathological MMR gene mutation, where applicable)	Surveillance interval (with corresponding MMR gene mutation, where applicable)	Comments
	National Comprehensive Cancer Network (NCCN) (45)	20-25 years (MLH1/ MSH2) 30-35 years (MSH6/ PMS2)	1-2 years 1-3 years	Offer colonoscopy 2-5 years younger than age of youngest diagnosed CRC case in family (if diagnosed before age 25)

carcinogenesis is less likely and has led to a switch of focus on optimising the colonoscopic procedure and adherence to key performance indictors for colonoscoy (10, 58–60).

3.1.1 Advanced imaging and artificial intelligence

High quality colonoscopy is crucial to the detection of both sporadic and hereditary CRC (61), especially in LS where lesions may be difficult to detect. To achieve this, different advanced imaging modalities including dye-based and virtual chromoendoscopy (VCE) have been assessed in patients with LS. A recent meta-analysis of four prospective studies comparing standard white light endoscopy (WLE) to chromoendoscopy using dye-spray showed that the latter was superior for detection of any adenomatous, flat, or proximal lesion (62). European guidelines suggest chromoendoscopy as an adjunct, whereas BSG guidelines advise that it offers no advantage to high-definition white light endoscopy (HDWLE) (10, 35, 36).

VCE is increasingly popular owing to its ease of use. Back-to-back studies comparing imaging modalities immediately following one another have shown a benefit for both narrow band imaging (NBI; Olympus) and iScan (Pentax) in LS polyp detection (63, 64). However, these comparisons have also shown higher lesion detection with dye-based chromoendoscopy versus NBI (65, 66). A recent multi-centre RCT compared HDWLE to Linked colour imaging (LCI; Fujifilm) among 357 patients with pathogenic LS variants and found no significant difference in polyp detection rate (44.4% vs. 36.0%; p=0.12) (67). Thus,at best, advanced imaging techniques can be an adjunct to HDWLE but cannot replace standard care.

In another growing field, the use of real-time artificial intelligence (AI)-colonoscopy has demonstrated enhanced detection of polyps and adenomas in average risk CRCs (68–71). A recent German RCT demonstrated a higher (albeit not statisticaly significant) rate of lesion detection, including LS-relevant flat lesions, by AI-colonoscopy than HDWLE in a LS cohort (72).

3.1.2 Non-invasive screening

A recent systemic review (73) brought attention to non-invasive biomarkers such as plasma-based methylated SEPTIN9, Big Adenine Tract-26 (a faceal marker of MSI), faecal sulfate-reducing bacteria *Desulfovibrio* and faecal immunochemical testing (FIT) in the detection of CRC and adenomas in LS, although further evidence is required to support their use in practice. A 2017 meta-analysis reported that FIT had a sensitivity of 85% for CRC and 46% for advanced adenomas in asymptomatic adults with a family history, suggesting that FIT alone would miss advanced neoplasia (74). However, during the COVID-19 pandemic in England, when access to non-urgent colonoscopy services was restricted, a temporary system based on FIT was introduced to risk stratify patients with LS to urgent colonoscopy (75). This formed the basis for an ongoing UK-based multi-centre prospective study examining a potential future role for FIT testing in LS (76).

3.2 Extra-colonic surveillance

Recommendations for the surveillance of LS-associated extra-colonic cancers are vary. For gastric cancers, most guidelines support routine testing for, and eradication of, *Helicobacter pylori*. American, Japanese and certain European guidelines advocate for regular oesophagogastroduodenoscopy (OGD) starting from 30-35 years of age (3, 37, 40, 77).

Beyond careful inspection of the duodenum and terminal ileum at OGD and colonoscopy respectively, routine testing for small bowel cancers is not typically recommended, though capsule endoscopy has been suggested for unexplained iron deficiency anaemia or abdominal pain (78).

LS families have been estimated to have an 8.6-fold increased risk of pancreatic cancer compared to the normal population (79) and surveillance using MRI or endoscopic ultrasound has been proposed for high-risk groups and carriers (80). However, low diagnostic yields and poor outcomes from surgical treatment of suspicious pancreatic lesions largely negate any theoretical benefit (10). Surveillance practices for LS-associated gynaecological cancers lack consensus and have not demonstrated a mortality benefit (81). American and European Oncology guidelines advocate for annual transvaginal ultrasound and endometrial sampling from the ages of 30-35, and prophylactic hysterectomy and bilateral salpingoophrectomy once child bearing completes, although the evidence for this is weak (3, 35, 37, 77). There is currently insufficient evidence to recommend screening for other extra-colonic LS cancers.

Unlike CRCs, for which standarised mortality ratios have been reported to decrease over time in LS cohorts, risk of death from LS-associated extra-colonic tumours is significantly increased compared with the general population (82). In a retrospective Finnish cohort, 7.2% of patients developed urothelial, prostate or

gastric cancer, with one in five dying from the disease (83). Extra-colonic surveillance may benefit those with cancer at a young age who have a higher lifetime risk of subsequent cancer, but this needs addressing in well-designed prospective trials.

4 Management

4.1 Preventative interventions

4.1.1 Modifiable risk factors

Most data on modifiable risk factors such as poor diet, high alcohol intake, smoking, lack of exercise and high body mass index (BMI) are extrapolated from sporadic CRC cohorts (84). Weak evidence specific to LS suggests lower CRC risk in patients who consume more fruit and higher risk in smokers (85). Subgroup analyses from the Colorectal Adenoma/Carcinoma Prevention Programme 2 (CAPP2) trial revealed a significant association between obesity and CRC risk (86). Two prospective cohort studies demonstrated a 30% increased risk of CRC for every 5.0 kg/m^2 increase in BMI in early adulthood and an association between an overweight BMI and CRC risk in men (87, 88).

4.1.2 Chemoprophylaxis

Aspirin is the only recommended chemoprophylaxis in LS. Its potential benefit was first highlighted by meta-analyses associating long-term use with lower incidence of all cancers, especially proximal CRC (89, 90). Subsequently the double-blinded RCT CAPP2, of 861 LS patients demonstrated that the use of 600mg/day of aspirin for 2-4 years was linked with a significantly lower risk of all LS-associated cancers after 10 year follow-up (91). A successive ongoing trial, CAPP3, aims to establish optimal dosing, meanwhile international guidelines have varied in their adoption of the CAPP2 findings. In the UK, both the BSG and NICE support the use of 150mg aspirin daily (300 mg if obese) in patients under 70 years old for 2-5 years (10, 92). American guidelines by contrast have refrained from recommending its use given data is currently derived from a single trial (3, 77).

4.2 Endoscopic and surgical management

Data on advanced endoscopic techniques to remove early-stage colorectal tumours in LS is lacking, therefore current practice heavily favours surgical resection. Endoscopic management follows guidance for non-LS colorectal polyps (93). As such, it is critical to optimise complete resection rates in LS-associated polypectomies, particularly for flat serrated polyps (94, 95).

The role of surgery in LS-associated CRC is two-fold: to resect the advanced neoplastic lesion and reduce the risk of metachronous disease. Meta-analyses have demonstrated a lower incidence of metachronous CRC in those who underwent extended resection (total/subtotal colectomy with ileorectal/ileosigmoidal anastomosis) versus segmental resection for a first CRC (96, 97) with absolute risk for metachronous tumour of 4.7% and 22.4%, respectively, over 100.7 months follow-up (98).

The risk of metachronous disease applies mainly to *MHL1* and *MSH2* pathogenic variant carriers and thus, in this context, most guidelines recommend the use of extended colectomy for a first CRC, particularly in younger patients (3, 10, 35, 37). For carriers of *MSH6* and *PMS2* variants there is insufficient evidence of oncological benefit to support the same approach, thus,for a first CRC, UK guidelines consider the two surgeries equal (10), whereas European guidelines advocate segmental resection unless there is a metachronous CRC (35).

4.3 Oncological management

4.3.1 Chemotherapy

Systemic anti-cancer treatment options for LS-CRCs were previously confined to the four chemotherapeutic agents used in sporadic CRCs (fluorouracil, leucovorin, oxaliplatin and irinotecan) with no consideration given to MSI or MMR status. Studies that explored the efficacy of these treatments in MSI-high CRCs were conflicting, not specific to LS and limited by small sample sizes (99–102). A single LS-CRC-specific retrospective study found no survival benefit associated with adjuvant fluorouracil (103). Nevertheless these agents remain in use as adjuvant treatment for some high-risk or late stage MSI-H/dMMR CRCs, both sporadic and LS-associated (104).

4.3.2 Immunotherapy

MMR-deficient CRCs demonstrate higher levels of immunogenicity than their MMR-proficient counterparts. MMR deficiency allows accumulation of point mutations in microsatellite sequences which can cause translational frameshifts, generating carboxy-terminal frameshift peptides (FSPs) that serve as "neoantigens" recognised by and stimulating the anti-tumour host immune response. The immunoreactive nature of MSI-high/dMMR CRCs prompted use of checkpoint inhibitors. The phase three KEYNOTE-177 trial demonstrated that pembrolizumab (anti-PD1) doubles the median progression-free survival compared to standard chemotherapy (16.5 vs 8.2 months) (105). As such, pembrolizumab is now approved by the USA Food and Drug Administration and recommended first-line treatment in the UK for metastatic MSI-high/dMMR CRCs. A second PD-1 inhibitor, nivolumab, is also NICE-approved for combination use with ipilimumab following standard combination chemotherapy (106).

It remains unknown whether LS-CRCs and sporadic MSI-high/dMMR CRCs share a common response to checkpoint inhibitor therapy. The higher neoantigen load in LS-CRCs might suggest an even more pronounced response, but available studies of checkpoint inhibitors that include LS patients are largely limited by small subgroup numbers and have not demonstrated a difference in response rates (107–110).

4.3.3 Vaccines

The compelling evidence for interplay between host immune surveillance and LS tumours has provided the conceptual basis for the use of vaccines to augment the adaptive immune response in LS. The high burden of foreign FSPs in LS makes them excellent vaccine

targets (111, 112). Although not specifically tested in LS-CRC, FSP-based vaccination induced significant humoral and T-cell responses in a first-in-human, phase I/IIa clinical trial (113) as well as in a mouse model of conditional *MSH2* knockout (114). The same principles underpin the use of cancer vaccines to prevent tumour development from premalignant polyps by targeting CRC-associated antigens such as MUC1 and CEA, a theory currently being tested and with promising results in mouse models (115, 116).

5 Conclusion

Lynch syndrome is encountered by many clinicians at some stage in their practice and yet remains under-diagnosed with historically limited success in risk stratification and management. The PLSD international database continues to expand our knowledge of LS-associated cancer risk. However, we have yet to obtain international consensus on the optimal surveillance strategies, which will be essential among a population of patients who are living beyond their index cancer. The advent of NGS into clinical practice will undoubtably improve detection rates and allow for more effective, precise, and personalised management programmes for patients with LS. Finally, over the next decade it will be exciting to see improvements in the preventative strategies that can be offered to patients in the form of aspirin, or even anti-cancer vaccines, as we continue to attempt to disrupt the natural history of this prevalent cancer predisposition syndrome.

Author contributions

MW, AH, BN, RK and LL contributed to conception and design of the review. MW, AH and BN performed literature review and wrote the first draft of the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Curtius K, Gupta S, Boland CR. Review article: lynch syndrome-a mechanistic and clinical management update. *Aliment Pharmacol Ther* (2022) 55(8):960–77. doi: 10.1111/apt.16826

2. Worthley DL, Leggett BA. Colorectal cancer: molecular features and clinical opportunities. *Clin Biochem Rev* (2010) 31(2):31–8.

3. Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW, et al. ACG clinical guideline: genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol* (2015) 110(2):223–62. doi: 10.1038/ajg.2014.435

4. Jasperson KW, Tuohy TM, Neklason DW, Burt RW. Hereditary and familial colon cancer. *Gastroenterology* (2010) 138(6):2044–58. doi: 10.1053/j.gastro.2010.01.054

5. Patel AP, Wang M, Fahed AC, Mason-Suares H, Brockman D, Pelletier R, et al. Association of rare pathogenic DNA variants for familial hypercholesterolemia, hereditary breast and ovarian cancer syndrome, and lynch syndrome with disease risk in adults according to family history. *JAMA Netw Open* (2020) 3(4):e203959. doi: 10.1001/jamanetworkopen.2020.3959

6. Dominguez-Valentin M, Sampson JR, Seppala TT, Ten Broeke SW, Plazzer JP, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the prospective lynch syndrome database. *Genet Med* (2020) 22(1):15–25. doi: 10.1038/s41436-019-0596-9

7. Hegde M, Ferber M, Mao R, Samowitz W, Ganguly A. Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee. ACMG technical standards and guidelines for genetic testing for inherited colorectal cancer (Lynch syndrome, familial adenomatous polyposis, and MYH-associated polyposis). *Genet Med* (2014) 16(1):101–16. doi: 10.1038/gim.2013.166

8. Peltomaki P. Update on lynch syndrome genomics. *Fam Cancer* (2016) 15 (3):385–93. doi: 10.1007/s10689-016-9882-8

9. Hitchins MP. Inheritance of epigenetic aberrations (constitutional epimutations) in cancer susceptibility. *Adv Genet* (2010) 70:201–43. doi: 10.1016/B978-0-12-380866-0.60008-3

10. Monahan KJ, Bradshaw N, Dolwani S, Desouza B, Dunlop MG, East JE, et al. Guidelines for the management of hereditary colorectal cancer from the British society of gastroenterology (BSG)/Association of coloproctology of great Britain and Ireland (ACPGBI)/United kingdom cancer genetics group (UKCGG). *Gut* (2020) 69(3):411–44. doi: 10.1136/gutjnl-2019-319915

11. NICE. *Molecular testing strategies for lynch syndrome in people with colorectal cancer. NICE diagnostic guidelines*. (2017).

12. Pearlman R, Frankel WL, Swanson BJ, Jones D, Zhao W, Yilmaz A, et al. Prospective statewide study of universal screening for hereditary colorectal cancer: the Ohio colorectal cancer prevention initiative. *JCO Precis Oncol* (2021) 5:779–91. doi: 10.1200/PO.20.00525

13. Burn J, Monahan K, Lalloo F, Peters K, Hardy S, Jessop J, et al *Implementing lynch syndrome testing and surveillance pathways*. NHS England (2021).

14. NICE. Testing strategies for lynch syndrome in people with endometrial cancer. *NICE Diagnostic Guidance* (2020). Available at: https://www.nice.org.uk/guidance/dg42

15. Vasen HF, Watson P, Mecklin JP, Lynch HTInternational Collaborative Group on HNPCC. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, lynch syndrome) proposed by the international collaborative group on HNPCC. *Gastroenterology* (1999) 116(6):1453–6. doi: 10.1016/S0016-5085(99)70510-X

16. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* (2004) 96(4):261–8. doi: 10.1093/jnci/djh034

17. Syngal S, Fox EA, Eng C, Kolodner RD, Garber JE. Sensitivity and specificity of clinical criteria for hereditary non-polyposis colorectal cancer associated mutations in MSH2 and MLH1. *J Med Genet* (2000) 37(9):641–5. doi: 10.1136/jmg.37.9.641

18. NICE. *Molecular testing strategies for lynch syndrome in people with colorectal cancer. NICE diagnostic guidelines*. (2022).

19. Yamamoto H, Imai K. An updated review of microsatellite instability in the era of next-generation sequencing and precision medicine. *Semin Oncol* (2019) 46(3):261–70. doi: 10.1053/j.seminoncol.2019.08.003

20. Gray PN, Tsai P, Chen D, Wu S, Hoo J, Mu W, et al. TumorNext-Lynch-MMR: a comprehensive next generation sequencing assay for the detection of germline and somatic mutations in genes associated with mismatch repair deficiency and lynch syndrome. *Oncotarget* (2018) 9(29):20304–22. doi: 10.18632/oncotarget.24854

21. Salvador MU, Truelson MRF, Mason C, Souders B, LaDuca H, Dougall B, et al. Comprehensive paired Tumor/Germline testing for lynch syndrome: bringing resolution to the diagnostic process. *J Clin Oncol* (2019) 37(8):647–57. doi: 10.1200/JCO.18.00696

22. Plazzer JP, Sijmons RH, Woods MO, Peltomäki P, Thompson B, Den Dunnen JT, et al. The InSiGHT database: utilizing 100 years of insights into lynch syndrome. *Fam Cancer* (2013) 12(2):175–80. doi: 10.1007/s10689-013-9616-0

23. European Hereditary Tumour Group. The prospective lynch syndrome database. (2023). Available at: https://www.ehtg.org/

24. Moller P. The prospective lynch syndrome database reports enable evidence-based personal precision health care. *Hered Cancer Clin Pract* (2020) 18:6. doi: 10.1186/s13053-020-0138-0

25. Moller P, Seppala TT, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the prospective lynch syndrome database. *Gut* (2018) 67(7):1306–16. doi: 10.1136/gutjnl-2017-314057

26. International Mismatch Repair Consortium. Variation in the risk of colorectal cancer in families with lynch syndrome: a retrospective cohort study. *Lancet Oncol* (2021) 22(7):1014–22. doi: 10.1016/S1470-2045(21)00189-3

27. Møller P, Seppälä T, Dowty JG, Haupt S, Dominguez-Valentin M, Sunde L, et al. Colorectal cancer incidences in lynch syndrome: a comparison of results from the prospective lynch syndrome database and the international mismatch repair consortium. *Hered Cancer Clin Pract* (2022) 20:36. doi: 10.1186/s13053-022-00241-1

28. Seppala TT, Dominguez-Valentin M, Sampson JR, Møller P. Prospective observational data informs understanding and future management of lynch syndrome: insights from the prospective lynch syndrome database (PLSD). *Fam Cancer* (2021) 20(1):35–9. doi: 10.1007/s10689-020-00193-2

29. Jarvinen HJ, Mecklin JP, Sistonen P. Screening reduces colorectal cancer rate in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology* (1995) 108(5):1405–11. doi: 10.1016/0016-5085(95)90688-6

30. Jarvinen HJ, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomäki P, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. *Gastroenterology* (2000) 118(5):829–34. doi: 10.1016/S0016-5085(00)70168-5

31. Newton K, Green K, Lalloo F, Evans DG, Hill J. Colonoscopy screening compliance and outcomes in patients with lynch syndrome. *Colorectal Dis* (2015) 17(1):38–46. doi: 10.1111/codi.12778

32. Stupart DA, Goldberg PA, Algar U, Ramesar R. Surveillance colonoscopy improves survival in a cohort of subjects with a single mismatch repair gene mutation. *Colorectal Dis* (2009) 11(2):126–30. doi: 10.1111/j.1463-1318.2008.01702.x

33. Lindberg LJ, Rasmussen M, Andersen KK, Nilbert M, Therkildsen C. Benefit from extended surveillance interval on colorectal cancer risk in lynch syndrome. *Colorectal Dis* (2020) 22(5):529–36. doi: 10.1111/codi.14926

34. Wales CINS. *Cancer genetics referral guidelines for colorectal cancer or polyposis risk assessment and consideration of genetic testing*. (2022).

35. Seppala TT, Latchford A, Negoi I, Sampaio Soares A, Jimenez-Rodriguez R, et al. European Guidelines from the EHTG and ESCP for lynch syndrome: an updated third edition of the mallorca guidelines based on gene and gender. *Br J Surg* (2021) 108(5):484–98. doi: 10.1002/bjs.11902

36. van Leerdam ME, Roos VH, van Hooft JE, Balaguer F, Dekker E, Kaminski MF, et al. Endoscopic management of lynch syndrome and of familial risk of colorectal cancer: European society of gastrointestinal endoscopy (ESGE) guideline. *Endoscopy* (2019) 51(11):1082–93. doi: 10.1055/a-1016-4977

37. Stjepanovic N, Moreira L, Carneiro F, Balaguer F, Cervantes A, Balmaña J, et al. Hereditary gastrointestinal cancers: ESMO clinical practice guidelines for diagnosis, treatment and follow-updagger. *Ann Oncol* (2019) 30(10):1558–71. doi: 10.1093/annonc/mdz233

38. French National Authority for Health. *Colorectal Cancer Screening and Prevention: Update of the Periodic Health Examination (PHA) Code of Practice*. (2013). Available at: https://www.has-sante.fr/jcms/c_1623732/en/depistage-et-prevention-du-cancer-colorectal

39. Hüneburg R, Aretz S, Büttner R, Daum S, Engel C, Fechner G, et al. Current recommendations for surveillance, risk reduction and therapy in lynch syndrome patients. *Z Gastroenterol* (2019) 57(11):1309–20. doi: 10.1055/a-1008-9827

40. Ishida H, Yamaguchi T, Tanakaya K, Akagi K, Inoue Y, Kumamoto K, et al. Japanese Society for cancer of the colon and rectum (JSCCR) guidelines 2016 for the clinical practice of hereditary colorectal cancer (Translated version). *J Anus Rectum Colon* (2018) 2(Suppl I):S1–S51. doi: 10.23922/jarc.2017-028

41. Integrated Cancer Center Netherlands. *Hereditary colorectal cancer*. (2014).

42. Guillen-Ponce C, Lastra E, Lorenzo-Lorenzo I, Martín Gómez T, Morales Chamorro R, Sánchez-Heras AB, et al. SEOM clinical guideline on hereditary colorectal cancer (2019). *Clin Transl Oncol* (2020) 22(2):201–12. doi: 10.1007/s12094-019-02272-y

43. Giardiello FM, Allen JI, Axilbund JE, Boland CR, Burke CA, Burt RW, et al. Guidelines on genetic evaluation and management of lynch syndrome: a consensus statement by the US multi-society task force on colorectal cancer. *Am J Gastroenterol* (2014) 109(8):1159–79. doi: 10.1038/ajg.2014.186

44. Stoffel EM, Mangu PB, Gruber SB, Hamilton SR, Kalady MF, Lau MW, et al. 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. *J Clin Oncol* (2015) 33(2):209–17. doi: 10.1200/JCO.2014.58.1322

45. Gupta S, Provenzale D, Llor X, Halverson AL, Grady W, Chung DC, et al. NCCN Guidelines Insights: Genetic/Familial High-Risk Assessment: Colorectal, Version 2.2019. *J Natl Compr Canc Netw* 17(9):1032–41. doi: 10.6004/jnccn.2016.0108

46. Pan JY, Haile RW, Templeton A, Macrae F, Qin F, Sundaram V, et al. Worldwide practice patterns in lynch syndrome diagnosis and management, based on data from the international mismatch repair consortium. *Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc* (2018) 16(12):1901. doi: 10.1016/j.cgh.2018.04.025

47. Moller P, Seppala T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Cancer incidence and survival in lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective lynch syndrome database. *Gut* (2017) 66(3):464–72. doi: 10.1136/gutjnl-2015-309675

48. Ten Broeke SW, van Bavel TC, Jansen AML, Gómez-García E, Hes FJ, van Hest LP, et al. Molecular background of colorectal tumors from patients with lynch syndrome associated with germline variants in PMS2. *Gastroenterology* (2018) 155(3):844–51. doi: 10.1053/j.gastro.2018.05.020

49. Ahadova A, Seppälä TT, Engel C, Gallon R, Burn J, Holinski-Feder E, et al. The "unnatural" history of colorectal cancer in lynch syndrome: lessons from colonoscopy surveillance. *Int J Cancer* (2021) 148(4):800–11. doi: 10.1002/ijc.33224

50. Moller P, Seppala T, Bernstein I, Holinski-Feder E, Sala P, Evans DG, et al. Incidence of and survival after subsequent cancers in carriers of pathogenic MMR variants with previous cancer: a report from the prospective lynch syndrome database. *Gut* (2017) 66(9):1657–64. doi: 10.1136/gutjnl-2016-311403

51. Seppala T, Pylvanainen K, Evans DG, Järvinen H, Renkonen-Sinisalo L, Bernstein I, et al. Colorectal cancer incidence in path MLH1 carriers subjected to different follow-up protocols: a prospective lynch syndrome database report. *Hered Cancer Clin Pract* (2017) 15(1):18. doi: 10.1186/s13053-017-0078-5

52. Engel C, Vasen HF, Seppala T, Aretz S, Bigirwamungu-Bargeman M, de Boer SY, et al. No difference in colorectal cancer incidence or stage at detection by colonoscopy among 3 countries with different lynch syndrome surveillance policies. *Gastroenterology* (2018) 155(5):1400–9 e2. doi: 10.1053/j.gastro.2018.07.030

53. Seppala TT, Ahadova A, Dominguez-Valentin M, Macrae F, Evans DG, Therkildsen , et al. Lack of association between screening interval and cancer stage in lynch syndrome may be accounted for by over-diagnosis; a prospective lynch syndrome database report. *Hered Cancer Clin Pract* (2019) 17:8. doi: 10.1186/s13053-019-0106-8

54. Lund J, Scholefield J, Grainge M, Smith SJ, Mangham C, Armitage NC, et al. Risks, costs, and compliance limit colorectal adenoma surveillance: lessons from a randomised trial. *Gut* (2001) 49(1):91. doi: 10.1136/gut.49.1.91

55. Edelstein DL, Axilbund J, Baxter M, Hylind LM, Romans K, Griffin CA, et al. Rapid development of colorectal neoplasia in patients with lynch syndrome. *Clin Gastroenterol Hepatol* (2011) 9(4):340–3. doi: 10.1016/j.cgh.2010.10.033

56. Iino H, Simms L, Young J, Arnold J, Winship IM, Webb SI, et al. DNA Microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. *Gut* (2000) 47(1):37–42. doi: 10.1136/gut.47.1.37

57. Ahadova A, von Knebel Doeberitz M, Bläker H, Kloor M. CTNNB1-mutant colorectal carcinomas with immediate invasive growth: a model of interval cancers in lynch syndrome. *Fam Cancer* (2016) 15(4):579–86. doi: 10.1007/s10689-016-9899-z

58. Sanchez A, Roos VH, Navarro M, Pineda M, Caballol B, Moreno L, et al. Quality of colonoscopy is associated with adenoma detection and postcolonoscopy colorectal cancer prevention in lynch syndrome. *Clin Gastroenterol Hepatol* (2022) 20(3):611–21.e9. doi: 10.1016/j.cgh.2020.11.002

59. Haanstra JF, Vasen HF, Sanduleanu S, van der Wouden EJ, Koornstra JJ, Kleibeuker JH, et al. Quality colonoscopy and risk of interval cancer in lynch syndrome. *Int J Colorectal Dis* (2013) 28(12):1643–9. doi: 10.1007/s00384-013-1745-2

60. Rees CJ, Thomas Gibson S, Rutter MD, Baragwanath P, Pullan R, Feeney M, et al. UK Key performance indicators and quality assurance standards for colonoscopy. *Gut* (2016) 65(12):1923–9. doi: 10.1136/gutjnl-2016-312044

61. Bisschops R, East JE, Hassan C, Hazewinkel Y, Kamiński MF, Neumann H, et al. Advanced imaging for detection and differentiation of colorectal neoplasia: European society of gastrointestinal endoscopy (ESGE) guideline - update 2019. *Endoscopy* (2019) 51(12):1155–79. doi: 10.1055/a-1031-7657

62. Har-Noy O, Yung DE, Koulaouzidis A, Eliakim R, Kopylov U, Avidan B, et al. Chromoendoscopy or white light endoscopy for neoplasia detection in lynch syndrome, a meta-analysis. *Dig Liver Dis* (2019) 51(11):1515–21. doi: 10.1016/j.dld.2019.07.018

63. Bisschops R, Tejpar S, Willekens H, De Hertogh G, Van Cutsem E. Virtual chromoendoscopy (I-SCAN) detects more polyps in patients with lynch syndrome: a randomized controlled crossover trial. *Endoscopy* (2017) 49(4):342–50. doi: 10.1055/s-0042-121005

64. East JE, Suzuki N, Stavrinidis M, Guenther T, Thomas HJ, Saunders BP. Narrow band imaging for colonoscopic surveillance in hereditary non-polyposis colorectal cancer. *Gut* (2008) 57(1):65–70. doi: 10.1136/gut.2007.128926

65. Huneburg R, Lammert F, Rabe C, Rahner N, Kahl P, Büttner R, et al. Chromocolonoscopy detects more adenomas than white light colonoscopy or narrow band imaging colonoscopy in hereditary nonpolyposis colorectal cancer screening. *Endoscopy* (2009) 41(4):316–22. doi: 10.1055/s-0028-1119628

66. Cellier C, Perrod G, Colas C, Dhooge M, Saurin JC, Lecomte T, et al. Back-to-Back comparison of colonoscopy with virtual chromoendoscopy using a third-generation narrow-band imaging system to chromoendoscopy with indigo carmine in patients with lynch syndrome. *Am J Gastroenterol* (2019) 114(10):1665–70. doi: 10.14309/ajg.0000000000000386

67. Houwen B, Hazewinkel Y, Pellise M, Rivero-Sánchez L, Balaguer F, Bisschops R, et al. Linked colour imaging for the detection of polyps in patients with lynch syndrome: a multicentre, parallel randomised controlled trial. *Gut* (2022) 71(3):553–60. doi: 10.1136/gutjnl-2020-323132

68. Gong D, Wu L, Zhang J, Mu G, Shen L, Liu J, et al. Detection of colorectal adenomas with a real-time computer-aided system (ENDOANGEL): a randomised controlled study. *Lancet Gastroenterol Hepatol* (2020) 5(4):352–61. doi: 10.1016/S2468-1253(19)30413-3

69. Su JR, Li Z, Shao XJ, Ji CR, Ji R, Zhou RC, et al. Impact of a real-time automatic quality control system on colorectal polyp and adenoma detection: a prospective randomized controlled study (with videos). *Gastrointest Endosc* (2020) 91(2):415–24.e4. doi: 10.1016/j.gie.2019.08.026

70. Wang P, Berzin TM, Glissen Brown JR, Bharadwaj S, Becq A, Xiao X, et al. Real-time automatic detection system increases colonoscopic polyp and adenoma detection rates: a prospective randomised controlled study. *Gut* (2019) 68(10):1813–9. doi: 10.1136/gutjnl-2018-317500

71. Repici A, Spadaccini M, Antonelli G, Correale L, Maselli R, Galtieri PA, et al. Artificial intelligence and colonoscopy experience: lessons from two randomised trials. *Gut* (2022) 71(4):757–65. doi: 10.1136/gutjnl-2021-324471

72. Hüneburg R, Bucksch K, Schmeisser F, Heling D, Marwitz T, Aretz S, et al. Real-time use of artificial intelligence (CADEYE) in colorectal cancer surveillance of patients with lynch syndrome-a randomized controlled pilot trial (CADLY). *United Eur Gastroenterol J* (2022) 11(1):60–8. doi: 10.1002/ueg2.12354

73. van Liere E, de Boer N, Dekker E, van Leerdam M, de Meij T, Ramsoekh D, et al. Systematic review: non-endoscopic surveillance for colorectal neoplasia in individuals with lynch syndrome. *Aliment Pharmacol Ther* (2022) 55(7):778–88. doi: 10.1111/apt.16824

74. Katsoula A, Paschos P, Haidich AB, Tsapas A, Giouleme O. Diagnostic accuracy of fecal immunochemical test in patients at increased risk for colorectal cancer: a meta-analysis. *JAMA Intern Med* (2017) 177(8):1110–8. doi: 10.1001/jamainternmed.2017.2309

75. Anne G Lincoln SCB, Sasieni P, Monahan KJ. Risk-stratified FIT for urgent colonoscopy in lynch syndrome: a clinical service throughout the COVID-19 pandemic. *J Clin Oncol* (2022) 40(16). doi: 10.1200/JCO.2022.40.16_suppl.10606

76. Lincoln A, Benton S, Piggott C, North BV, Rigney J, Young C, et al. Exploring the utility and acceptability of faecal immunochemical testing (FIT) as a novel intervention for the improvement of colorectal cancer (CRC) surveillance in individuals with lynch syndrome (FIT for lynch study): a single-arm, prospective, multi-centre, non-randomised study. *BMC Cancer* (2022) 22(1):1144. doi: 10.1186/s12885-022-10217-y

77. Stoffel EM, Mangu PB, Limburg PJ, Hamilton SR, Kalady MF, Lau MW, et al. 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. *J Oncol Pract* (2015) 11(3):e437–41. doi: 10.1200/JOP.2015.003665

78. ten Kate GL, Kleibeuker JH, Nagengast FM, Craanen M, Cats A, Menko FH, et al. Is surveillance of the small bowel indicated for lynch syndrome families? *Gut* (2007) 56(9):1198–201. doi: 10.1136/gut.2006.118299

79. Kastrinos F, Mukherjee B, Tayob N, Wang F, Sparr J, Raymond VM, et al. Risk of pancreatic cancer in families with lynch syndrome. *JAMA* (2009) 302(16):1790–5. doi: 10.1001/jama.2009.1529

80. Canto MI, Harinck F, Hruban RH, Offerhaus GJ, Poley JW, Kamel I, et al. International cancer of the pancreas screening (CAPS) consortium summit on the management of patients with increased risk for familial pancreatic cancer. *Gut* (2013) 62(3):339–47. doi: 10.1136/gutjnl-2012-303108

81. Crosbie EJ, Ryan NAJ, Arends MJ, Bosse T, Burn J, Cornes JM, et al. The Manchester international consensus group recommendations for the management of gynecological cancers in lynch syndrome. *Genet Med* (2019) 21(10):2390–400. doi: 10.1038/s41436-019-0489-y

82. Evans DG, Ingham SL. Reduced life expectancy seen in hereditary diseases which predispose to early-onset tumors. *Appl Clin Genet* (2013) 6:53–61. doi: 10.2147/TACG.S35605

83. Pylvanainen K, Lehtinen T, Kellokumpu I, Järvinen H, Mecklin JP. Causes of death of mutation carriers in Finnish lynch syndrome families. *Fam Cancer* (2012) 11(3):467–71. doi: 10.1007/s10689-012-9537-3

84. Islami F, Goding Sauer A, Miller KD, Siegel RL, Fedewa SA, Jacobs EJ, et al. Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the united states. *CA Cancer J Clin* (2018) 68(1):31–54. doi: 10.3322/caac.21440

85. Diergaarde B, Braam H, Vasen HF, Nagengast FM, van Muijen GN, Kok FJ, et al. Environmental factors and colorectal tumor risk in individuals with hereditary nonpolyposis colorectal cancer. *Clin Gastroenterol Hepatol* (2007) 5(6):736–42. doi: 10.1016/j.cgh.2007.02.019

86. Movahedi M, Bishop DT, Macrae F, Mecklin JP, Moeslein G, Olschwang S, et al. Obesity, aspirin, and risk of colorectal cancer in carriers of hereditary colorectal cancer: a prospective investigation in the CAPP2 study. *J Clin Oncol* (2015) 33(31):3591–7. doi: 10.1200/JCO.2014.58.9952

87. Botma A, Nagengast FM, Braem MG, Hendriks JC, Kleibeuker JH, Vasen HF, et al. Body mass index increases risk of colorectal adenomas in men with lynch syndrome: the GEOLynch cohort study. *J Clin Oncol* (2010) 28(28):4346–53. doi: 10.1200/JCO.2010.28.0453

88. Win AK, Dowty JG, English DR, Campbell PT, Young JP, Winship I, et al. Body mass index in early adulthood and colorectal cancer risk for carriers and non-carriers of germline mutations in DNA mismatch repair genes. *Br J Cancer* (2011) 105(1):162–9. doi: 10.1038/bjc.2011.172

89. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet* (2011) 377(9759):31–41. doi: 10.1016/S0140-6736(10)62110-1

90. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet* (2010) 376(9754):1741–50. doi: 10.1016/S0140-6736(10)61543-7

91. Burn J, Sheth H, Elliott F, Reed L, Macrae F, Mecklin JP, 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(10240):1855–63. doi: 10.1016/S0140-6736(20)30366-4

92. NICE. *Colorectal cancer. NICE clinical guideline NG151*. (2020).

93. Ferlitsch M, Moss A, Hassan C, Bhandari P, Dumonceau JM, Paspatis G, et al. Colorectal polypectomy and endoscopic mucosal resection (EMR): European society of gastrointestinal endoscopy (ESGE) clinical guideline. *Endoscopy* (2017) 49(3):270–97. doi: 10.1055/s-0043-102569

94. Suzuki S, Ikehara H, Gotoda T. Should large sessile serrated lesions be treated with cold snare polypectomy? *Dig Endosc* (2022) 34(3):485–7. doi: 10.1111/den.14257

95. Takeuchi Y, Shichijo S, Uedo N, Kawakami Y, Okubo Y, Tani Y, et al. Safety and efficacy of cold versus hot snare polypectomy including colorectal polyps >/=1 cm in size. *Dig Endosc* (2022) 34(2):274–83. doi: 10.1111/den.14096

96. Heneghan HM, Martin ST, Winter DC. Segmental vs extended colectomy in the management of hereditary nonpolyposis colorectal cancer: a systematic review and meta-analysis. *Colorectal Dis* (2015) 17(5):382–9. doi: 10.1111/codi.12868

97. Anele CC, Adegbola SO, Askari A, Rajendran A, Clark SK, Latchford A, et al. Risk of metachronous colorectal cancer following colectomy in lynch syndrome: a systematic review and meta-analysis. *Colorectal Dis* (2017) 19(6):528–36. doi: 10.1111/codi.13679

98. Malik SS, Lythgoe MP, McPhail M, Monahan KJ. Metachronous colorectal cancer following segmental or extended colectomy in lynch syndrome: a systematic review and meta-analysis. *Fam Cancer* (2018) 17(4):557–64. doi: 10.1007/s10689-017-0062-2

99. Fallik D, Borrini F, Boige V, Viguier J, Jacob S, Miquel C, et al. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. *Cancer Res* (2003) 63(18):5738–44.

100. Carethers JM, Smith EJ, Behling CA, Nguyen L, Tajima A, Doctolero RT, et al. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* (2004) 126(2):394–401. doi: 10.1053/j.gastro.2003.12.023

101. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* (2003) 349(3):247–57. doi: 10.1056/NEJMoa022289

102. Liang JT, Huang KC, Lai HS, Lee PH, Cheng YM, Hsu HC, et al. High-frequency microsatellite instability predicts better chemosensitivity to high-dose 5-fluorouracil plus leucovorin chemotherapy for stage IV sporadic colorectal cancer after palliative bowel resection. *Int J Cancer* (2002) 101(6):519–25. doi: 10.1002/ijc.10643

103. de Vos tot Nederveen Cappel WH, Meulenbeld HJ, Kleibeuker JH, Nagengast FM, Menko FH, Griffioen G, et al. Survival after adjuvant 5-FU treatment for stage III colon cancer in hereditary nonpolyposis colorectal cancer. *Int J Cancer* (2004) 109(3):468–71. doi: 10.1002/ijc.11712

104. Argiles G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, et al. Localised colon cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* (2020) 31(10):1291–305. doi: 10.1016/j.annonc.2020.06.022

105. Diaz LAJr., Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, et al. Pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomised, open-label, phase 3 study. *Lancet Oncol* (2022) 23(5):659–70. doi: 10.1016/S1470-2045(22)00197-8

106. NICE. *Nivolumab with ipilimumab for previously treated metastatic colorectal cancer with high microsatellite instability or mismatch repair deficiency. technology appraisal guidance*. (2021).

107. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* (2015) 372 (26):2509–20. doi: 10.1056/NEJMoa1500596

108. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* (2017) 357(6349):409–13. doi: 10.1126/science.aan6733

109. Overman MJ, Lonardi S, Wong KYM, Lenz HJ, Gelsomino F, Aglietta M, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-Deficient/Microsatellite instability-high metastatic colorectal cancer. *J Clin Oncol* (2018) 36(8):773–9. doi: 10.1200/JCO.2017.76.9901

110. Therkildsen C, Jensen LH, Rasmussen M, Bernstein I. An update on immune checkpoint therapy for the treatment of lynch syndrome. *Clin Exp Gastroenterol* (2021) 14:181–97. doi: 10.2147/CEG.S278054

111. Hernandez-Sanchez A, Grossman M, Yeung K, Sei SS, Lipkin S, Kloor M. Vaccines for immunoprevention of DNA mismatch repair deficient cancers. *J Immunother Cancer* (2022) 10(6). doi: 10.1136/jitc-2021-004416

112. Schwitalle Y, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, et al. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. *Gastroenterology* (2008) 134(4):988–97. doi: 10.1053/j.gastro.2008.01.015

113. Kloor M, Reuschenbach M, Pauligk C, Karbach J, Rafiyan MR, Al-Batran SE, et al. A frameshift peptide neoantigen-based vaccine for mismatch repair-deficient cancers: a phase I/IIa clinical trial. *Clin Cancer Res* (2020) 26(17):4503–10. doi: 10.1158/1078-0432.CCR-19-3517

114. Gebert J, Gelincik O, Oezcan-Wahlbrink M , Marshall JD, Hernandez-Sanchez A, Urban K, et al. Recurrent frameshift neoantigen vaccine elicits protective immunity with reduced tumor burden and improved overall survival in a lynch syndrome mouse model. *Gastroenterology* (2021) 161(4):1288–302.e13. doi: 10.1053/j.gastro.2021.06.073

115. Zeytin HE, Patel AC, Rogers CJ, Canter D, Hursting SD, Schlom J, et al. Combination of a poxvirus-based vaccine with a cyclooxygenase-2 inhibitor (celecoxib) elicits antitumor immunity and long-term survival in CEA.Tg/MIN mice. *Cancer Res* (2004) 64(10):3668–78. doi: 10.1158/0008-5472.CAN-03-3878

116. Greiner JW, Zeytin H, Anver MR, Schlom J. Vaccine-based therapy directed against carcinoembryonic antigen demonstrates antitumor activity on spontaneous intestinal tumors in the absence of autoimmunity. *Cancer Res* (2002) 62(23):6944–51.

Frontiers in Oncology

Advances knowledge of carcinogenesis and tumor progression for better treatment and management

The third most-cited oncology journal, which highlights research in carcinogenesis and tumor progression, bridging the gap between basic research and applications to imrpove diagnosis, therapeutics and management strategies.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact