

# ERN GENTURIS GUIDELINE ON CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY DIAGNOSIS, GENETIC COUNSELLING, SURVEILLANCE, QUALITY OF LIFE, AND CLINICAL MANAGEMENT

*Publication date 17 October 2024*

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## Version control/ document history

Issue Date	Version	Changes Made / Reason for this issue
17.10.2024	Public version	Public version, paper in European Journal of Human Genetics online: <a href="https://doi.org/10.1038/s41431-024-01708-6">doi.org/10.1038/s41431-024-01708-6</a>
22.01.2024	Final version	Approved by ERN GENTURIS Board Members. The ERN GENTURIS Board comprises the 1st representatives of the ERN GENTURIS Full Members (Full Members are indicated with an asterisk in the acknowledgement below), and the representative of the ERN GENTURIS ePAG Patient Council.

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**Acknowledgement:** We would like to thank Manon Engels and Jurriaan Hölzenspies for logistic support, coordination of the guideline committee meetings and facilitating the guideline development process, the ERN Guidelines consortium - AQuAS for the literature review and evidence synthesis, European care for CMMRD (C<sub>4</sub>CMMRD) consortium for reaching out to their members to participate in the guideline Group and the Delphi, and all Delphi participants (listed in chapter 8.1) for their participation in the Delphi survey.

We acknowledge all healthcare providers participating in ERN GENTURIS: Medical University of Innsbruck, Innsbruck, Austria; Medical University of Vienna, Vienna, Austria; University Hospital Brussels, Brussels, Belgium\*; Ghent University Hospital, Ghent, Belgium\*; University Hospital Leuven, Leuven, Belgium\*; University Hospital Liege, Liege, Belgium\*; Motol University Hospital, Prague, Czech Republic\*; Masaryk Memorial Cancer Institute, Brno, Czech Republic\*; Archbishop Makarios III Hospital, Karaiskakio Foundation, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; Aarhus Universitetshospital, Aarhus, Denmark\*; Rigshospitalet, Copenhagen, Denmark\*; Tartu University Hospital,

Tartu, Estonia; GENTURISFINSN consortium (HUS Helsinki University Hospital and OYS Oulu University Hospital), Helsinki, Finland\*; The Hospital District of Southwest Finland, Turku University Hospital, Turku, Finland\*; University Hospital Henri Mondor-National Referral Center, Créteil, France\*; Institut Curie, Paris, France\*; Rouen University Hospital, Rouen, France\*; Center for Hereditary Tumor Syndromes (CHT), University Hospital Bonn, Bonn, Germany\*; Hereditary Cancer Syndrome Center Dresden, Dresden, Germany\*; Universitätsklinikum Erlangen, Erlangen, Germany\*; Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany\*; Medizinische Hochschule Hannover, Hannover, Germany\*; Medizinisch Genetisches Zentrum, Munich, Germany\*; Aghia Sophia Children's Hospital, Athens, Greece\*; University of Pécs, Pécs, Hungary\*; Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy\*; Fondazione IRCCS CA'Granda Ospedale Maggiore Policlinico, Milano, Italy\*; Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy\*; Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy\*; Azienda Ospedaliera di Padova, Padua, Italy\*; Azienda Ospedaliero-Universitaria di Parma, Parma, Italy\*; Fondazione Policlinico Universitario A. Gemelli, Rome, Italy\*; IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy\*; Azienda Ospedaliero-Universitaria Senese, Siena, Italy\*; Pauls Stradins Clinical University Hospital, Riga, Latvia\*; Vilniaus universiteto ligoninės Santaros klinikos, Vilnius, Lithuania\*; Centre Hospitalier du Luxembourg, Luxembourg, Luxembourg; Mater Dei Hospital, Msida, Malta; Netherlands Cancer Institute - Antoni van Leeuwenhoek, Amsterdam, the Netherlands\*; University Medical Center Groningen, Groningen, the Netherlands\*; Leiden University Medical Center, Leiden, the Netherlands\*; Radboud university medical center, Nijmegen, the Netherlands\*; Erasmus Medical Center, Rotterdam, the Netherlands\*; Haukeland University Hospital, Bergen, Norway; Pomeranian Medical University - University Clinical Hospital no 1, Szczecin, Poland\*; Instituto Português de Oncologia de Lisboa Francisco Gentil, EPE, Lisbon, Portugal\*; Centro Hospitalar Universitário de São João – CHUSJ, Porto, Portugal\*; Porto Comprehensive Cancer Center, Porto, Portugal\*; Institute of Oncology Ljubljana, Ljubljana, Slovenia\*; Hospital Germans Trias I Pujol - Institut Català d'Oncologia, Barcelona, Spain\*; Hospital Sant Joan de Déu, Barcelona, Spain\*; Karolinska University Hospital, Stockholm, Sweden\*.

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I, Katharina Wimmer, sign off the document "CONFIDENTIAL\_20240116\_CMMRD guideline document\_final" on 21-01-2024, for distribution to the ERN GENTURIS network board for their approval.

I, Chrystelle Colas, have signed off the document "CONFIDENTIAL\_20240116\_CMMRD guideline document\_final" on 18-01-2024, for distribution to the ERN GENTURIS network board for their approval.

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## TABLE OF CONTENTS

1. Abstract.....	7
2. Guideline Summary.....	9
3. Introduction.....	11
4. Composition of the guideline group .....	16
5. Conflict of interests .....	18
6. Purpose and Scope of this guideline .....	19
6.1 Why was this guideline produced? .....	19
6.2 Who is the guideline for? .....	19
6.3 What is the guideline about? .....	20
6.3.1 Scope.....	20
6.3.2 Health Questions .....	20
6.3.3 Population .....	21
6.3.4 Care setting.....	22
6.3.5 Aetiology & Epidemiology .....	22
7. Recommendations .....	26
7.1 Diagnosis Recommendations .....	26
7.2 Genetic counselling recommendations.....	34
7.3 Surveillance Recommendations .....	35
7.4 Quality of Life Recommendations .....	39
7.5 Clinical management Recommendations .....	39
8. Methods for Guideline Development.....	42
8.1 Formulating and grading statements and consensus building .....	42
8.2 Internal and External review .....	49
8.3 Timeline and procedure for updating the guideline .....	49
8.4 Funding and Financial support.....	50
9. Summary of Evidence and Recommendations .....	51
9.1 Diagnosis - summary of evidence and guideline recommendations.....	51
9.2 Genetic counselling - summary of evidence and guideline recommendations .....	68
9.3 Surveillance - summary of evidence and guideline recommendations.....	72
9.4 Quality of life - summary of evidence and guideline recommendations.....	86
9.5 Clinical management - summary of evidence and guideline recommendations.....	88
10. What do other guidelines state? .....	100
11. Suggestions for future research.....	107
12. Glossary.....	113
13. Bibliography.....	116

## 1. ABSTRACT

Constitutional mismatch repair deficiency (CMMRD), caused by bi-allelic germline pathogenic variants in one of four MMR genes, is associated with an extremely high cancer risk starting from early childhood and throughout life. The CMMRD-associated tumour spectrum includes primarily hematologic, brain, and gastrointestinal tract malignancies. The majority of CMMRD patients also have distinctive non-neoplastic features.

Specific tumour and family history characteristics, and non-neoplastic features are important clinical indicators for CMMRD testing. However, the phenotypic overlap with other cancer predisposition syndromes and limitations of genetic testing may prevent an early and unequivocal diagnosis. Our understanding of the CMMRD phenotype has improved in recent years and several assays assessing a hallmark feature of CMMRD, microsatellite instability in non-neoplastic blood leukocytes, have been developed that circumvent the diagnostic limitations of genetic testing. Existing guidelines for the clinical suspicion of CMMRD need updating and integration into a comprehensive framework for CMMRD diagnosis. Previously suggested surveillance protocols need adjustment to take into account recent observational prospective studies, which have shown the effectiveness of surveillance for the detection of brain and gastrointestinal tract tumours but not haematologic malignancies. Malignant gliomas and intestinal tract cancers have been shown to be responsive to immune checkpoint inhibitors (ICI). These findings and the effectiveness and toxicity of radiotherapy, thiopurines, methylating agents, and other chemotherapeutics, need to be incorporated in comprehensive guidelines.

Method: A Guideline Group with members of multiple specialties and one patient representative developed recommendations for CMMRD *diagnosis, genetic counselling, surveillance, quality of life, and clinical management* based on a thorough literature review. Two rounds of a modified Delphi process were used to gain maximal consensus for approval of the recommendations. Experts in this exercise included the 19 members of the Guideline Group including members of the European Reference Network GENTURIS and/or the European care for CMMRD (C4CMMRD) consortium, as well as 53 additional external experts identified by the Guideline Group.

Results: Fourteen recommendations for the *diagnosis* of CMMRD provide indication criteria for CMMRD testing based on (updated) existing C4CMMRD clinical guidelines for paediatric/young adult cancer patients and for genetically unconfirmed suspected sporadic NF1 patients as well as on tumour characteristics indicative for CMMRD. Further recommendations define the criteria for a CMMRD diagnosis and testing strategies.

Twelve recommendations for *genetic counselling* include recommendations for predictive testing in relatives, for prenatal and preimplantation CMMRD testing and for MMR gene analysis in partners of CMMRD and Lynch syndrome patients.

Twenty-nine recommendations for *surveillance* include specific recommendations concerning brain tumour surveillance by MRI and lower and upper gastrointestinal tract surveillance by colonoscopy and (video capsule) endoscopy. Recommendations for lymphoma, leukaemia, gynaecological and urinary tract cancer surveillance and for whole body MRI are given. All recommendations contain information on the starting age, the frequency, and modalities of the surveillance methods.

Four recommendations for *quality of life* mainly address psychological support and age adapted patient and family education.

Twenty-three recommendations for *clinical management* consist of general recommendations on malignancy treatment (including radiation therapy and stem cell transplantation), a large proportion of them with only moderate or low evidence due to the paucity of data. Specific recommendations are given for ICI therapy of high-grade glioma, colorectal cancer, other Lynch-related and non-Lynch related malignancies and for chemotherapy of non-Hodgkin lymphoma and leukaemia. Management of polyposis, low-grade glioma, medulloblastoma, suspected tumour relapse and IgG/A production deficits, surveillance during tumour treatment, and colorectal cancer prevention with acetylsalicylic acid are topics also covered.

Conclusions: Based on existing guidelines and currently available data, we defined 82 recommendations for the care of patients with CMMRD. These guidelines do not consider specifics of countries with low-resources or with entirely private health care systems. These recommendations are not meant to be prescriptive and may be adjusted based on individual decisions made, wherever possible, in multidisciplinary boards and after discussion with CMMRD experts as well as with the patient and/or their family.



## 2. GUIDELINE SUMMARY

This guideline has been drawn from the best available evidence and the consensus of experts in this area. It is regularly updated to reflect changes in evidence. The expectation is that clinicians will follow this guideline unless there is a compelling clinical reason to undertake different management, specific to an individual patient.

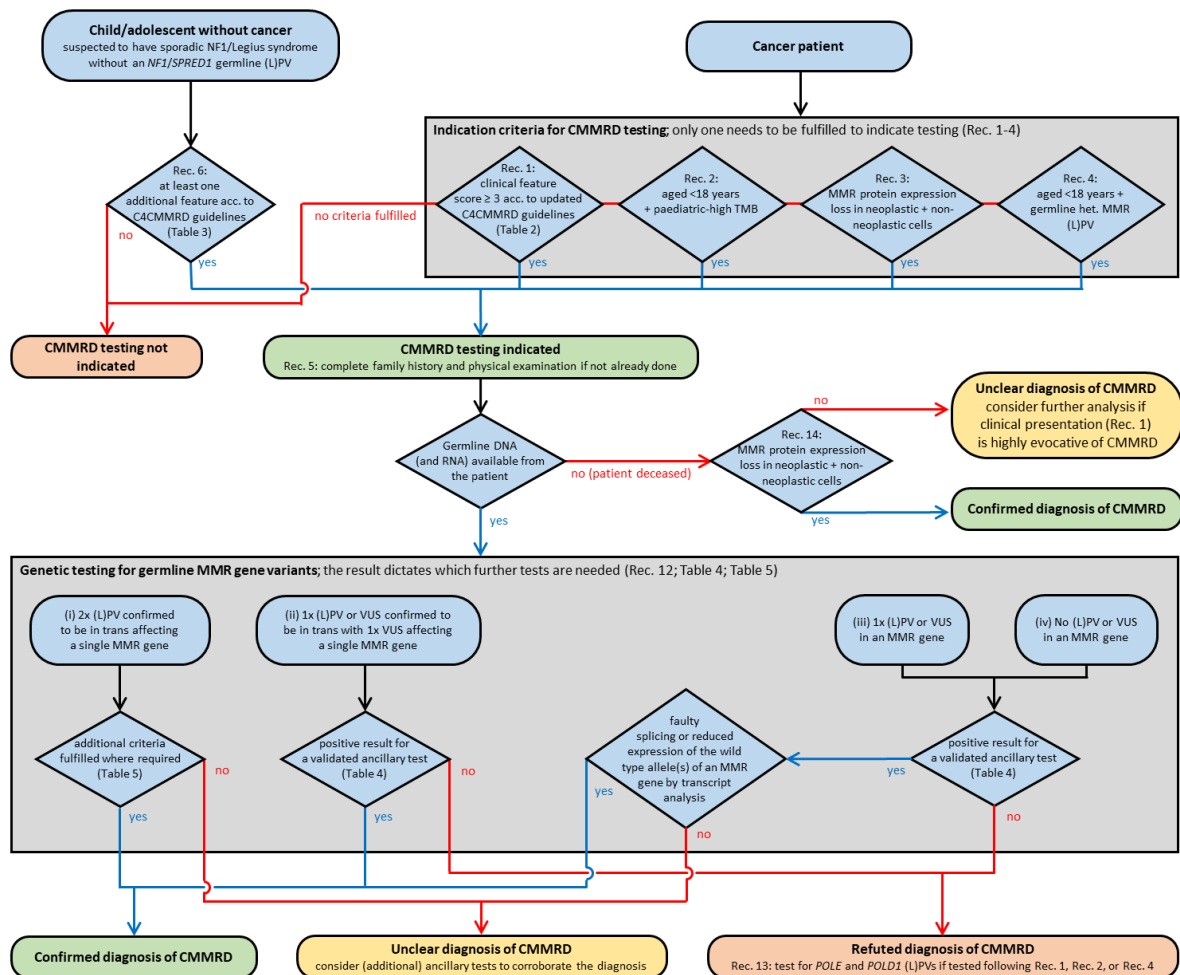


Figure 1. Decision tree for the diagnosis of CMMRD

**Table 1. Summary of surveillance recommendations**

Exam		Frequency	Period	Evidence*
Clinical examination		Every 6 months	From diagnosis	Strong
Brain MRI		Every 6 months	2 – 20 years	Strong
		Annually	From 20 years	Moderate
Colonoscopy		Annually**	From 6 years	Strong
Upper gastrointestinal endoscopy		Annually**	Simultaneously with colonoscopy or at least from age 10 years	Strong
Video capsule endoscopy		Annually	From 10 years	Strong
Gynaecologic	surveillance (clinical examination & transvaginal ultrasound)	Annually	From 20 years	Strong
	Prophylactic surgery	Not applicable	Discuss once family planning is completed	Moderate
Abdominopelvic ultrasound for gynaecological and urinary tract cancer screening		Annually	From 20 years	Strong
Whole body MRI		At least once	At diagnosis or when anaesthesia is no longer required	Strong
		Discuss optional annual imaging		Moderate

\*This grading is based on published articles and expert consensus.

\*\* Interval should be increased to once every 6 months once polyps are detected

### 3. INTRODUCTION

Mismatch repair (MMR) is a cellular mechanism involved in the repair of replication errors that escape proofreading by the exonuclease activity of the replicative DNA polymerases  $\epsilon$  and  $\delta$  (Pol  $\epsilon$  and Pol  $\delta$ ) (Jiricny, 2006). MMR deficiency resulting from inactivation of one of four MMR genes (*MLH1*, MIM# 120436; *MSH2*, MIM# 609309; *MSH6*, MIM# 600678; *PMS2*, MIM# 600259) leads to mutation accumulation in dividing cells and cancer (Zou et al., 2021). Constitutional (hereafter referred to as germline) pathogenic variants (PVs) in one of these four genes are associated with an increased risk of cancers involving multiple organs. Individuals with a heterozygous germline MMR gene PV have Lynch syndrome (LS), an autosomal dominant, adult-onset cancer predisposition syndrome. The LS cancer spectrum includes mainly colorectal and endometrial carcinoma, as well as cancers of other organs at a lower frequency, such as the small bowel, the urinary tract, the stomach, the ovaries and the brain. In LS associated cancers, MMR deficiency results from somatic inactivation of the wild-type allele of the germline mutated MMR gene. In 1999, two reports described the phenotype of offspring from consanguineous parents within LS families who carried homozygous *MLH1* germline PVs and, hence, a constitutional MMR deficiency (CMMRD). They developed haematological malignancies and a brain tumour in one individual, and displayed clinical features reminiscent of neurofibromatosis type 1 (NF1) (Ricciardone et al., 1999; Wang et al., 1999). Since then, well over 200 paediatric and young adult patients have been reported carrying biallelic germline PV in one of the four MMR genes. This recessively inherited condition is now recognised as a distinct childhood cancer predisposition syndrome (OMIM #276300) named CMMRD syndrome or simply CMMRD. Other names used previously include biallelic Mismatch Repair Deficiency (bMMRD), Mismatch Repair Cancer Syndrome (MMRCS) or Brain Tumour Polyposis Syndrome (BTPS<sub>1</sub>). Although molecularly not proven, it is retrospectively most likely that Jacques Turcot in 1959 described the first cases of CMMRD when he reported two siblings with numerous colorectal adenomatous polyps, colorectal carcinoma and malignant brain tumours, and so historically CMMRD cases have also been described as Turcot syndrome (Turcot et al., 1959).

The cancer risk in CMMRD is one of the highest – if not the highest - among (childhood) cancer syndromes. Individuals with CMMRD have a substantial risk of developing cancer

from their first year of life and thereafter, and it is extremely uncommon for a patient to have no tumour diagnosis by their third decade of life (Vasen et al., 2014; Wimmer et al., 2014). Without surveillance, patients often die before adulthood (Durno et al., 2021; Ghorbanoghli et al., 2023). The known spectrum of CMMRD-associated cancers is broad and, in essence, any cancer could be associated with CMMRD. The three most frequent cancer groups are (i) haematological malignancies diagnosed in ~40% of patients, with T-cell lymphoblastic lymphoma being most prevalent, (ii) malignant brain tumours in ~55% of patients, most frequently high-grade gliomas (HGGs), and, (iii) colorectal carcinomas (CRC) and other LS-associated tumours in ~50% of patients. Most patients develop digestive tract adenoma ranging from a single adenoma (often with high-grade dysplasia) to polyposis in the second decade of life. It is likely that any patient who is not under colorectal surveillance and reaches adolescence or young adulthood will eventually develop CRC. Tumour types less frequently seen in CMMRD include neuroblastoma, nephroblastoma, different types of sarcomas, and others (Durno et al., 2017a; Durno et al., 2021; Johannesma et al., 2011; Kratz et al., 2009; Lavoine et al., 2015; Levi et al., 2015; Ripperger & Schlegelberger, 2016; Wimmer et al., 2014). Overall, patients with biallelic *MLH1/MSH2* PV show a more severe phenotype with an earlier age at first tumour than those with biallelic *MSH6* and *PMS2* mutations (Bruekner et al., 2023; Wimmer et al., 2014). Attenuated forms of CMMRD also exist. The few reported cases have been linked to hypomorphic MMR variants and are characterised by a lower frequency of both haematological and brain malignancies as well as a later onset of cancer (Bruekner et al., 2023; Gallon et al., 2024; Li et al., 2015).

In childhood or adolescent cancer patients, the tumour type, additional non-malignant neoplasia such as colorectal polyps, family history, and the presence of non-neoplastic manifestations of CMMRD are important clinical indicators to raise suspicion of a CMMRD diagnosis (Wimmer et al., 2014). The most prevalent of these non-neoplastic features are café-au-lait maculae (CALMs) reminiscent of NF1 (Wimmer et al., 2017b), other hypo- and hyper-pigmented skin patches (Wimmer et al., 2017b), and multiple developmental venous anomalies (DVAs) (Raveneau et al., 2024; Shiran et al., 2018; Wimmer et al., 2017b). A scoring system was formulated from these CMMRD-associated clinical features to provide clear criteria to select patients for germline genetic testing (Wimmer et al., 2014).

Over 60% of CMMRD cases result from biallelic *PMS2* PVs, over 20% from biallelic *MSH6* PVs, and less than 20% from either *MLH1* PVs or *MSH2* PVs (Ercan et al., 2024; Wimmer et al., 2014). These numbers reflect the estimated population prevalence of heterozygous PV in these four MMR genes (Win et al., 2017). The birth incidence is approximately one in a million if parents are not related (Perez-Valencia et al., 2020; Suerink et al., 2019a). However, the prevalence might be higher in populations with founder mutations and/or a high rate of parental consanguinity. Indeed, approximately half of CMMRD cases are homozygous for a MMR gene PV (Lavoine et al., 2015; Wimmer et al., 2014). Although both parents of a CMMRD patient are obligate carriers of a monoallelic MMR gene PV, a family history of LS associated cancers is often lacking (Lavoine et al., 2015) as the penetrance of monoallelic *MSH6* and especially *PMS2* PVs is substantially lower than that of *MSH2* and *MLH1* PVs, which account for the majority of LS cancer patients (Dominguez-Valentin et al., 2020; Moller et al., 2018; Suerink et al., 2019b).

Most of the variants found in CMMRD patients are truncating and so expected to cause complete loss of expression and, consequently, function of the corresponding MMR protein. However, almost 30% of those variants have been described as variant of uncertain significance (VUS) (more frequently in *MSH2* and *MLH1* than in *PMS2* and *MSH6*) (Bodo et al., 2015). This high rate of VUS and the difficulties of *PMS2* analysis resulting from the presence of the *PMS2CL* pseudogene (Ganster et al., 2010; Hayward et al., 2007; Mandelker et al., 2016; van der Klift et al., 2010), complicates diagnosis and has led to development of ancillary tests that can confirm or exclude the diagnosis in patients in whom genetic testing leads to an inconclusive result (Bodo et al., 2015; Chung et al., 2021; Chung et al., 2023; Gallon et al., 2019; Gallon et al., 2023; Gonzalez-Acosta et al., 2020; Ingham et al., 2013).

Due to the high tumour risk and broad tumour spectrum, CMMRD patients need to be subjected to extensive surveillance and several protocols for surveillance have been proposed (Durno et al., 2017a; Tabori et al., 2017; Vasen et al., 2014). In observational prospective studies, these protocols have proven to be effective for brain and digestive tract

tumours, but not for haematological malignancies (Durno et al., 2021; Ghorbanoghli et al., 2023).

CMMRD cancers are inherently MMR deficient and this shapes tumour molecular pathology. MMR deficient cancers have increased tumour mutational burden (TMB) and microsatellite instability (MSI) as substitution and small insertion-deletion mutations generated during DNA replication are not repaired. They are frequently classified as hypermutated, which is typically defined as a TMB  $\geq 10$  mutations per megabase (mut/Mb). In CMMRD brain tumours, it is also common to find concurrent polymerase proofreading deficiency caused by missense variants in the exonuclease domains of replicative DNA polymerases Pol  $\epsilon$  or Pol  $\delta$ . This results in ultramutated tumours with TMB  $\geq 100$  mut/Mb (Andrianova et al., 2017; Shlien et al., 2015; Waterfall & Meltzer, 2015). A high TMB is associated with response to immunotherapy through translation of coding variants producing tumour-specific, immunogenic neoantigens (Sha et al., 2020). MMR deficient tumours may also be particularly responsive to immunotherapy as they produce highly immunogenic frameshift peptides (FSPs). Insertion-deletion variants in coding microsatellites accumulate in MMR deficient cancers, which create shifts in gene reading frames. When translated, these frameshift mutations can generate long stretches of novel amino acid sequence – FSPs – whereas point mutations create only single amino acid substitutions. FSPs can therefore harbour many epitopes for immune cell receptor binding during antigen presentation, and so more opportunity for immune cell activation than peptides containing substitution variants (Kloor & von Knebel Doeberitz, 2016). It follows that immune checkpoint inhibitors (ICIs) are a promising drug class in the treatment of CMMRD cancers, with clinical responses being observed in gastrointestinal and brain tumours (Das et al., 2022; Suerink et al., 2021a). MMR deficiency also confers therapy-resistance to tumours, in particular against chemotherapies that rely on functional MMR for their mechanism of action. The inefficacy of temozolomide to treat MMR deficient brain tumours is of particular note for CMMRD patients (Gan et al., 2022).

Current guidelines for the diagnosis of CMMRD and CMMRD cancer surveillance protocols have been published from two expert groups, the European consortium Care for CMMRD

(C<sub>4</sub>CMMRD) and the International Replication Repair Deficiency Consortium (IRRDC) along with collaborating health care organisations (Aronson et al., 2022; Durno et al., 2017a; Suerink et al., 2018; Tabori et al., 2017; Vasen et al., 2014; Wimmer et al., 2014). Recent developments in diagnosis, in particular improved understanding of the CMMRD clinical phenotype and the development of low cost and scalable assays to detect MSI in blood as a pathognomonic feature of CMMRD (Chung et al., 2023; Gallon et al., 2023), and the efficacy of cancer surveillance protocols (Durno et al., 2021; Ghorbanoghli et al., 2023) could not be considered in earlier guidelines. Professional guidelines on genetic counselling, quality of life, and cancer treatment are also lacking, and CMMRD healthcare practice is varied. Therefore, up to date and comprehensive guideline recommendations for the diagnosis and management of CMMRD are needed.

## 4. COMPOSITION OF THE GUIDELINE GROUP

The European Reference Network (ERN) Guideline Group for people with CMMRD was established by clinical geneticists and clinicians with expertise in CMMRD, as well as an affected individual. These guidelines were written for healthcare practitioners caring for CMMRD patients, but can be used by any interested party.

The CMMRD Guideline Group was supported by a Core Working Group, including ERN GENTURIS healthcare provider members from different Member States and members of the European C4CMMRD consortium, who are recognised experts and specialised in different aspects of the diagnosis and management of CMMRD. The Core Working Group met online monthly and drafted the guideline scope, clinical questions, recommendations and guideline document and obtained feedback from the CMMRD Guideline Group. The recommendations were finalised in a modified Delphi approach in which the Core Working Group, CMMRD Guideline Group (including patient representative) and additional experts participated (see chapter 8).

### **Approach to secure views and preference of target population**

The ERN GENTURIS CMMRD Guideline Group was supported by a patient representative who has experience with CMMRD. This patient representative was part of the core working group and present during several of these meetings.

Involving the patient representative in the development of these guidelines and in the Guideline Group helped to ensure that:

- The questions addressed are relevant to them and will make a positive impact on patient care.
- Important aspects of the experience of illness are considered.
- Critical clinical and patient important outcomes are identified and prioritised.
- The balance of benefits and harms of the intervention is appropriately considered, when recommendations are formulated in conjunction with patient values and preferences.



The patient representative advised on the scope, target population and clinical questions the guideline aimed to address and provided feedback on the plain language summary and patient journey.

## 5. CONFLICT OF INTERESTS

All members of the ERN GENTURIS CMMRD Guideline Group, including the Core Working Group, have provided disclosure statements on all relationships that they have that might be perceived to be a potential conflict of interest. Amedeo Azizi and Chrystelle Colas report receipt of honoraria or consultation fees from AstraZeneca. Kevin Beccaria reports previous employment with Carthera SAS. Laurence Brugières reports receipts of honoraria or consultation fees from ESAI and TAKEDA. Volodia Dangouloff-Ros reports receipt of grants/research support from GE Healthcare. Richard Gallon reports receipt of grants/research support from Cancer Research UK Catalyst and UK National Health Service. Magali Svrcek reports receipt of grants/research support from Bayer and Roche, and receipt of honoraria or consultation fees from Astellas, MSD and Sanofi.

All participants of the ERN GENTURIS CMMRD Delphi survey have provided disclosure statements on all relationships that they have that might be perceived to be a potential source of a competing interests. Andishe Attarbaschi reports receipt of honoraria or consultation fees from Amgen, Novartis, Jazz, Gilead and MSD. Patrick Benusiglio reports receipt of honoraria or consultation fees from AstraZeneca, BMS and MSD. Christof Kramm reports receipt of grants/research support from Deutsche Kinderkrebsstiftung (non-commercial), research collaboration with Bayer to develop NTRK-inhibitors, being a member of the advisory board for Boehringer Ingelheim, and participation in Blueprint Medicines ROVER trial NCT04773782. Eric Legius reports receipt of honoraria or consultation fees from Alexion and AstraZeneca. Rianne Oostenbrink reports receipt of grants/research support from EU Patient-centric clinical trial platform (EU-PEARL), which includes support from the European Union's Horizon 2020 research and innovation program, EFPIA, Children's Tumor Foundation, Global Alliance for TB Drug Development non-profit organization and Springworks Therapeutics Inc., receipt of honoraria or consultation fees from AstraZeneca, and participation in a speaker's bureau sponsored by Alexion. Enrico Opocher reports receipt of honoraria or consultation fees from Alexion (RareDisease). Markus G. Seidel reports receipt of grants/research support from Takeda, Amgen and Novartis, and receipt of honoraria or consultation fees from Jazz, Amgen and Novartis.

## 6. PURPOSE AND SCOPE OF THIS GUIDELINE

### 6.1 WHY WAS THIS GUIDELINE PRODUCED?

Before this guideline, there were different and limited guideline recommendations for the diagnosis and management of CMMRD and there is substantial variability in clinical practice.

Currently, two main professional groups have set up an international cooperation in order to improve diagnosis and care of CMMRD patients: the European C<sub>4</sub>CMMRD consortium and the Canadian-led International Replication Repair Deficiency Consortium (IRRDC). In their name, together, or through collaborations (with the US Multi-Society Task Force on Colorectal Cancer and the Pediatric Cancer Working Group of the American Association for Cancer Research), they have published different guidelines regarding diagnosis and surveillance of CMMRD patients based on expert opinions and available data.

Since then, knowledge of the CMMRD phenotype and the suitability of different features as indicators for CMMRD testing have advanced, and new ancillary assays have been developed that complement genetic testing. Furthermore, observational prospective studies conducted by C<sub>4</sub>CMMRD and IRRDC have demonstrated a survival benefit for individuals with CMMRD who undergo surveillance. New data were also published on the efficacy of immunotherapy for treatment of CMMRD patient cancers and for the treatment of MMR deficient tumours in general.

It was desirable to update and merge the different guidelines on diagnosis and clinical management (surveillance and treatment) of CMMRD patients in one comprehensive guideline. There is a consensus that such a guideline is overdue to improve and standardise diagnosis and management for the benefit of CMMRD patients and their families. Therefore, ERN GENTURIS and the European consortium C<sub>4</sub>CMMRD undertook a common effort to define recommendations for the care of CMMRD in Europe and beyond.

### 6.2 WHO IS THE GUIDELINE FOR?

The CMMRD Guideline Group has prepared this guideline document to assist healthcare professionals in evidence-based diagnosis, genetic counselling, clinical management and surveillance of people with CMMRD.

Although the guidelines are written primarily for geneticists, paediatric haematologists-oncologists and gastroenterologists, they can also be used by other physicians, patients or other interested parties.

Clinical guidelines are statements to support decision making, based on systematically evaluated evidence for a specified clinical setting. Whilst these clinical guidelines are based on the latest published evidence, care of each individual remains primarily the responsibility of their treating medical professionals. Decisions for care should always be based on the needs, preferences and circumstances of each patient. Clinical guidelines should support clinical decision making, but never replace clinical professionals. Guidelines present recommendations based on expert opinion and published evidence and are not mandates. These guidelines do not signify nor intend to be a legal standard of care.

## 6.3 WHAT IS THE GUIDELINE ABOUT?

### 6.3.1 SCOPE

The scope of this guideline is to define the optimal diagnosis, surveillance, and clinical management of people with CMMRD. The genetic counselling section also addresses recommendations for their relatives.

### 6.3.2 HEALTH QUESTIONS

It is critical to define the key clinical questions regarding diagnosis, surveillance, and clinical management of people with CMMRD.

Key clinical questions include, but are not restricted, to:

#### **DIAGNOSIS**

What clinical and molecular criteria should be used to indicate a genetic test for the diagnosis of CMMRD?

Which technique is most favourable to use with regard to the performance, limitations, costs, availability, turnaround time, invasiveness and acceptance of the technique?

When is the diagnosis of CMMRD genetically confirmed and when can we clearly refute the diagnosis of CMMRD?

## **GENETIC COUNSELLING**

After a diagnosis of CMMRD in a patient, which people should receive genetic counselling within the family?

What kind of reproductive issues should be discussed with CMMRD patients and/or their parents?

Should the risk of having a child with CMMRD be discussed with LS carriers?

## **SURVEILLANCE**

In people with CMMRD, what would be the optimal screening method, starting age, and interval to detect each tumour type?

## **QUALITY OF LIFE**

What is the psychological impact of a CMMRD diagnosis and what psychosocial support do people with CMMRD benefit from?

## **CLINICAL MANAGEMENT**

If a tumour is diagnosed, is the treatment different in the context of CMMRD compared to the treatment of the same tumour in another context?

If it is different, what is the optimal, CMMRD-specific treatment?

### **6.3.3 POPULATION**

The target population for this guideline is all individuals with CMMRD, which mainly consist of children and young adults. Genetic counselling and genetic testing recommendations also consider family members, such as the parents with a high likelihood of having LS and patient's siblings and the reproductive partners of CMMRD and LS carriers.

#### 6.3.4 CARE SETTING

The guideline is intended to support the decision making of geneticists, clinical geneticists, (paediatric) oncologists, pathologists, molecular geneticists performing genetic testing, (paediatric) gastroenterologists, (paediatric) dermatologists, and radiologist, in their decisions on diagnosis, counselling, surveillance and clinical management of people with CMMRD. The guideline can also be used by other physicians (general doctors, psychologists and other specialists involved in CMMRD care), patients or other interested parties.

Implementation of this guideline will require dissemination to the different stakeholders. Preferably, this European guideline should be adopted and diffused by the Directorate-General of Health of each European Country. A more fragmented but rather more feasible approach will be to disseminate these guidelines via professional and patient societies. A concise version of these guidelines has been published in the European Journal of Human Genetics.

#### 6.3.5 AETIOLOGY & EPIDEMIOLOGY

##### **Aetiology**

The mismatch repair (MMR) system plays an important role in the correction of DNA replication errors during cell division. This includes the recognition of base-base mismatches and insertion/deletion loops (IDLs). IDLs occur frequently in microsatellites, tandem repeats of short (1-9 nucleotides) DNA sequence motifs (Jiricny, 2006). Loss of MMR function due to pathogenic variants in both copies of either the *MLH1*, *MSH2*, *MSH6* or *PMS2* gene significantly increases mutation rate up to hundreds-fold (Zou et al., 2021). This mutator phenotype can drive tumourigenesis through accumulation of additional mutations in oncogenes and tumour suppressor genes (Negrini et al., 2010). For example, in colorectal cancer (CRC), MMR deficient tumours have different spectra of mutated genes and mutation types compared to MMR proficient tumours, with an increase in frameshift mutations at coding microsatellites and C>T transitions (Ahadova et al., 2018; Cancer Genome Atlas, 2012; Sekine et al., 2017), mutations that are characteristic of MMR deficient mutational signatures (Alexandrov et al., 2020; Zou et al., 2021).

In Lynch syndrome (LS), patients are born with an MMR gene PV in one allele and their cells can become MMR deficient through acquisition of an inactivating second hit in the other, i.e. the wild-type, allele of the same gene. Hence, LS carriers have an increased risk of MMR deficient cancers (Latham et al., 2019). In CMMRD, caused by biallelic germline PV in one of these four MMR genes, all cells of the individual are MMR deficient and replication errors remain uncorrected from gestation onwards. The increased constitutional mutation rate of CMMRD is thought to explain its severe phenotype as well as the presence of constitutional microsatellite instability (Gallon et al., 2023; Zou et al., 2021). It is feasible that an increased constitutional mutation rate could be responsible for somatic mutations of non-MMR genes in otherwise normal tissues and causes some of the non-neoplastic clinical features of CMMRD, such as café au lait spots (CALS) and other alterations of skin pigmentation, and brain cavernomas (Alotaibi et al., 2008; Guerrini-Rousseau et al., 2024; Wimmer et al., 2017b).

Other functions of the MMR system may also influence the presentation of CMMRD. For example, the role of MMR in a signalling cascade that induces apoptosis upon DNA damage results in the resistance of CMMRD tumours to some chemotherapies, and its role in immunoglobulin class switch recombination causes abnormal immunoglobulin production in CMMRD patients (Li, 2008; Tesch et al., 2018). Primary immunodeficiency has been observed in rare cases, but a mechanistic association with CMMRD has not been proven (Peron et al., 2008).

## **Epidemiology**

The most commonly involved genes in CMMRD are *PMS2* and *MSH6* while biallelic *MLH1* and *MSH2* PVs are rarer. This distribution of biallelic PVs fits with the frequency of monoallelic PVs in these genes in the general population, in which *PMS2* and *MSH6* are about 2.5 - 4 times more prevalent than *MLH1/MSH2* PVs (Win et al., 2017). The overrepresentation of *MLH1* and *MSH2* PVs in clinically recognized Lynch syndrome patients reflects ascertainment that occurs in cancer patients referred for genetic testing, since penetrance of PVs in these

genes is higher than for *PMS2* and *MSH6* (Dominguez-Valentin et al., 2020; Goodenberger et al., 2016; Haraldsdottir et al., 2017; Ten Broeke et al., 2018). Furthermore, it might be speculated that an embryonic lethality of homozygous PVs in *MSH2* or *MLH1* may also account for the distribution difference in CMMRD.

Up to now, well over 200 genetically proven patients with CMMRD have been published. Based on the most recent empiric estimation of the carrier frequencies of *MLH1*, *MSH2*, *MSH6* and *PMS2* PVs (Win et al., 2017) the incidence of CMMRD in the general population was calculated to be one in a million children of unrelated parents (Suerink et al., 2019a). This estimate is supported also by an empirical study assessing the frequency of CMMRD patients among suspected NF1 children without malignancy who tested negative for an *NF1* or *SPRED1* PV (Perez-Valencia et al., 2020). However, it must be assumed that the incidence will be substantially higher in populations with founder MMR gene PVs and in children of consanguineous parents. Indeed, homozygosity for founder variants or consanguineous parentage were observed in 46/91 (50.5%) of CMMRD patients (Wimmer et al., 2014). This number is supported by a French cohort, in which approximately half of the CMMRD cases were homozygous for an MMR gene PV mainly due to parental consanguinity while the other half were compound heterozygous and from unrelated parents (Lavoine et al., 2015). Examples from specific populations have also been reported. Li and colleagues estimated that one in 16 individuals of the Nunavik Inuit population is a carrier for the *PMS2* c.2002A>G p.(Ile668\*) PV, leading to a high frequency of CMMRD (Li et al., 2015). Similarly, the Icelandic population has three MMR founder variants at relatively high frequencies: *PMS2* c.736\_741del6ins1 p.(Pro246Cysfs\*3), *PMS2* c.2T>A p.(Met1?), and *MSH6* c.1754T>C p.(Leu585Pro) with carrier frequencies of 0.234%, 0.092%, and 0.080%, respectively (Haraldsdottir et al., 2017), which could lead to a higher birth incidence of CMMRD than the estimated one in a million. A study of Jordanian paediatric/adolescent HGG patients found that approximately 30% of tumours showed loss of MMR protein expression in both tumour and normal cells, suggestive of a high CMMRD frequency, though this was not confirmed by genetic testing. This may be explained by the high frequency of consanguineous parentage in approximately 30% of the cohort (Amayiri et al., 2016).



Limited data is available on the prevalence of CMMRD in specific cancer subtypes and will, among other factors, depend on the strength of the association of the tumour type with CMMRD and the rarity of the specific tumour subtype during childhood and adolescence. A CMMRD prevalence of 9.1% (8/88) was reported in a national cohort of T-cell lymphoblastic lymphoma patients aged  $\leq 18$  years (Kroeze et al., 2022). Mork et al. found CMMRD in 1.0% (2/193) of young adult (age  $< 35$  years) CRC patients and de Voer et al. in 2.7% (2/74) of CRC patients aged younger than 25 years (de Voer et al., 2021; Mork et al., 2015). From a report of childhood/adolescent cancer patients, it can be deduced that CMMRD is associated with approximately 2.5% (3/118) of high grade glioma cases (Supp. Tables S1 & S7 in Gröbner et al., 2018). Finally, 7.4% (14/189) of childhood/adolescent non-Hodgkin lymphoma patients who have secondary malignant neoplasia were found to have CMMRD in one recent study (Attarbaschi et al., 2021).

## 7. RECOMMENDATIONS

Recommendations in this guideline are divided into 5 sections: diagnosis, genetic counselling, surveillance, quality of life, and clinical management

### 7.1 DIAGNOSIS RECOMMENDATIONS

Recommendations		Strength
<b>Rec. 1</b>	CMMRD testing should be offered to all cancer patients who reach a minimum of three scoring points according to the revised C4CMMRD indication criteria (Table 2).	Strong
<b>Rec. 2</b>	CMMRD testing should be offered to all cancer patients aged <18 years with a tumour that has a paediatric-high* tumour mutational burden (TMB), regardless of presence or absence of a somatic <i>POLE</i> or <i>POLD1</i> pathogenic variant. *(Gröbner et al., 2018; Merino et al., 2020)	Strong
<b>Rec. 3</b>	CMMRD testing should be offered to all cancer patients with a tumour that has expression loss of one or more of the four MMR proteins by immunohistochemical staining in neoplastic and in non-neoplastic cells including tumour infiltrating leukocytes and/or endothelial cells.	Strong
<b>Rec. 4</b>	CMMRD testing should be offered to all cancer patients aged <18 years in whom a heterozygous (likely) pathogenic variant in one of the MMR genes was found by germline sequencing.	Strong
<b>Rec. 5</b>	A family history assessment and physical examination should be performed for any patient who fulfils inclusion criteria of CMMRD testing as described in Rec. 2-4.	Strong

<b>Rec. 6</b>	CMMRD testing should probably be offered in expert centres, following an interdisciplinary discussion to all children suspected to have sporadic NF1/Legius syndrome without cancer and without an <i>NF1/SPRED1</i> germline (L)PV after comprehensive genetic analysis and who have at least one additional feature defined by the C4CMMRD guidelines (Suerink et al 2018, Table 3).	Strong
	<b>Testing strategy</b>	
<b>Rec. 7</b>	Any testing strategy should aim to come to a definite diagnosis that either confirms or refutes CMMRD in the patient, and to identify the causative variants in the relevant MMR gene.	Strong
<b>Rec. 8</b>	Wherever possible, CMMRD testing of a patient with a (pre-)malignancy should include immunohistochemical staining of all four MMR proteins in tumour tissue to determine MMR protein expression in neoplastic and in non-neoplastic cells, including tumour infiltrating leukocytes and/or endothelial cells.	Strong
<b>Rec. 9</b>	The laboratory performing genetic CMMRD testing should be able to offer transcript analysis of all four MMR genes and should be able to apply assays that circumvent potential diagnostic pitfalls that result from the high homology of <i>PMS2</i> and its pseudogene <i>PMS2CL</i> (either by partnership with a different laboratory or in their own laboratory).	Strong
<b>Rec. 10</b>	The laboratory performing genetic CMMRD testing of an index patient with a (pre-)malignancy should probably have one or more validated ancillary assay(s) available (either by partnership with a different laboratory or in their own laboratory) that can definitively confirm or refute the diagnosis of CMMRD if genetic testing renders an inconclusive result (the currently available	Strong

	ancillary assays testing for constitutional MMR deficiency are listed in Table 4).	
<b>Rec. 11</b>	The laboratory performing genetic CMMRD testing of an index patient without a (pre-)malignancy should have one or more validated ancillary assay(s) available (either by partnership with a different laboratory or in their own laboratory) that can definitively confirm or refute the diagnosis of CMMRD if genetic testing renders an inconclusive result (the currently available ancillary assays testing for constitutional MMR deficiency are listed in Table 4).	Strong
	<b>Diagnostic criteria</b>	
<b>Rec. 12</b>	<p>The diagnosis of CMMRD should be considered confirmed in an individual fulfilling one or more of the suggested criteria for CMMRD testing (Rec.1, Rec.2, Rec.3, Rec.4, Rec.6) if, according to the Table "Criteria for the confirmation of CMMRD" (Table 5):</p> <p>(i) in one of the four MMR genes, two variants classified according to internationally accepted classification criteria* as (likely) pathogenic (PV or LPV) are identified and are confirmed to be located in trans (note that in some cases additional criteria need to be fulfilled);</p> <p>OR</p> <p>(ii) in one of the four MMR genes, one of two variants identified and confirmed to be located in trans is classified as a PV or LPV or variant of unknown significance (VUS) and the other one is classified as a VUS and one or more clinically validated ancillary test results is consistent with a CMMRD diagnosis;</p> <p>OR</p>	Moderate

	<p>(iii) in one of the four MMR genes, one variant is identified and classified as a PV or LPV or VUS and there is evidence for (a) faulty splicing not explained by the identified variant or (b) reduced expression of the wild-type allele by transcript analysis and one or more clinically validated ancillary test results is consistent with a CMMRD diagnosis;</p> <p>OR</p> <p>(iv) no MMR gene variant classified as a PV or LPV or VUS is identified, but one or more clinically validated ancillary test results is consistent with a CMMRD diagnosis and there is evidence by transcript analysis for (a) faulty splicing or (b) reduced expression of the wild-type allele(s) of one of the MMR genes.</p> <p><a href="#">*ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for MMR genes.</a></p>	
<p><b>Rec. 13</b></p>	<p>Cancer patients fulfilling the suggested criteria for CMMRD testing, Rec.1, Rec.2 or Rec.4, in whom the diagnosis CMMRD cannot be confirmed, should probably be tested for a germline (likely) pathogenic variant in the exonuclease domains of <i>POLE</i> and <i>POLD1</i>.</p>	<p>Strong</p>
<p><b>Rec.14</b></p>	<p>In a deceased cancer patient fulfilling one or more of the suggested criteria for CMMRD testing (Rec.1, Rec.2, Rec.4) for whom no germline DNA/RNA is available and the diagnosis of CMMRD cannot be confirmed by one or more of the criteria outlined in Rec.12 and Table 5, the diagnosis of CMMRD should be considered confirmed if immunohistochemical staining shows</p>	<p>Moderate</p>

expression loss of one or more MMR proteins in neoplastic and in non-neoplastic cells, including tumour infiltrating leukocytes and/or endothelial cells, of the patient and expression in an appropriate positive control.
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**Table 2: Revised C<sub>4</sub>CMMRD indication criteria for CMMRD testing in cancer patients\***  
**CMMRD testing is indicated in a cancer patient reaching  $\geq 3$  points.**

<b><u>C<sub>4</sub>CMMRD scoring points assigned to (pre-)malignancies in the patient (at least one point is mandatory):</u></b>	
Carcinoma of the Lynch syndrome (LS) spectrum* and/or a high-grade dysplastic adenoma of the digestive tract at age <25 years	3 points
Multiple colorectal adenomas at age <25 years and no genetic diagnosis/explanation upon testing for polyposis syndromes	3 points
T-cell lymphoblastic lymphoma (T-LBL) at age <18 years	2 points
WHO grade III or IV glioma at age <25 years	2 points
Any other malignancy at age <18 years	1 point
<b><u>C<sub>4</sub>CMMRD scoring points assigned to additional features in the patient (optional):</u></b>	
Clinical sign of Neurofibromatosis type 1 (NF1) <sup>§</sup> and/or $\geq 4$ hyperpigmented and/or hypopigmented skin alterations with $\emptyset^{\#} > 1$ cm	2 points
2 or 3 hyperpigmented and/or hypopigmented skin alterations with $\emptyset > 1$ cm <b>Do not count if two points are already given for "Clinical sign of NF1 and/or <math>\geq 4</math> hyperpigmented and/or hypopigmented skin alterations with <math>\emptyset &gt; 1</math> cm"</b>	1 point
Multiple pilomatrixomas	2 points
One pilomatrixoma	1 point
Agenesis of the corpus callosum	1 point
Non-therapy-induced cavernoma	1 point
Multiple developmental venous anomalies (DVAs, also known as cerebral venous angiomas) in separate regions of the brain	2 points
Paediatric systemic lupus erythematosus	1 point
Deficiency/reduced levels of IgG2/4 and/or IgA	1 point
<b><u>C<sub>4</sub>CMMRD scoring points assigned to additional features in the family (optional):</u></b>	
Consanguineous parents	1 point
Diagnosis of LS in a first-degree or second-degree relative	2 points
Carcinoma from LS spectrum* before the age of 60 years in a first-degree, second-degree, and/or third-degree relative	1 point
A sibling with a (pre-)malignancy assigned two or three C <sub>4</sub> CMMRD scoring points	2 points
A sibling with any type of childhood malignancy	1 point

**Abbreviations:** C<sub>4</sub>CMMRD = Care for CMMRD; (L)PV(s) = (likely) pathogenic variant(s); WHO = World Health Organization; NF1 = neurofibromatosis type 1.

<sup>†</sup>Original C4CMMRD criteria: Wimmer 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.

\*Colorectal, endometrial, small bowel, urothelial, gastric, ovarian, and biliary tract cancer.

<sup>§</sup>Clinical sign in the patient used for the diagnosis of NF1 according to: Legius et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. Genet Med 2021; 23(8):1506-1513.

<sup>#</sup>Diameter

**Table 3: Selection strategy for CMMRD counselling and testing in a child suspected to have NF1/Legius syndrome (without cancer) and a negative outcome of NF1/SPRED1 germline mutation analysis**

Prerequisites:
<ul style="list-style-type: none"> <li>▶ Suspicion of NF1 due to the presence of at least one diagnostic NF1 feature<sup>*</sup>, including at least two hyperpigmented skin patches reminiscent of CALMs.</li> <li>▶ No (likely) pathogenic germline variant in <i>NF1</i> and <i>SPRED1</i> detected using comprehensive and highly sensitive mutation analysis protocols<sup>#</sup>.</li> <li>▶ Absence of diagnostic NF1 sign(s) in both parents.</li> </ul>
Additional features, at least one (either in the family or in the patient) is required:
<p>In the family:</p> <ul style="list-style-type: none"> <li>▶ Consanguineous parents.</li> <li>▶ Genetic diagnosis of Lynch syndrome in one or both parental families.</li> <li>▶ Sibling with diagnostic NF1 sign(s).</li> <li>▶ A (deceased) sibling<sup>§</sup> with any type of childhood malignancy.</li> <li>▶ One of the following carcinomas of the Lynch syndrome spectrum: Colorectal, endometrial, small bowel, urothelial, gastric, ovarian, and biliary tract cancer, before the age of 60 years in a first-degree or second-degree relative.</li> </ul>
<p>In the patient:</p> <ul style="list-style-type: none"> <li>▶ Atypical CALMs (irregular borders and/or pigmentation).</li> <li>▶ Multiple hypopigmented skin patches.</li> <li>▶ One or more pilomatrixoma(s) in the patient.</li> <li>▶ Agenesis of the corpus callosum.</li> <li>▶ Non-therapy-induced cavernoma.</li> <li>▶ Multiple developmental venous anomalies (also known as cerebral venous angiomas) in separate regions of the brain.</li> </ul>

**Abbreviations:** CMMRD - constitutional mismatch repair deficiency; NF1 - neurofibromatosis type 1; CALMs - café-au-lait macules.

<sup>\*</sup>Legius et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. Genet Med 2021; 23(8):1506-1513.

<sup>#</sup>Analysis protocol should include methods that identify and/or characterise unusual splice variants.

<sup>§</sup>This can be expanded to second-degree and third-degree relatives in populations with a high prevalence of founder mutations.

**Table 4: Ancillary tests for assessing constitutional MMR deficiency**

Validated test <sup>#</sup>	CMMRD confirmed	CMMRD refuted
Germline Microsatellite instability (gMSI) testing acc. to Ingham et al. 2013 <sup>a</sup>	gMSI ratios of at least two (usually all three) microsatellite markers are above the validated laboratory's internal thresholds	Not possible by the test
Constitutional MSI (cMSI) testing acc. to Gallon et al. 2019 and 2023 <sup>b</sup>	cMSI score above the validated laboratory's internal thresholds	cMSI score within the score range of negative controls
High-sensitivity MSI (hsMSI) testing acc. to González-Acosta et al. 2020 <sup>c</sup>	hsMSI score above the validated laboratory's internal thresholds	hsMSI score within the score range of negative controls
Ex vivo MSI (evMSI) + methylation tolerance acc. to Bodo et al. 2015 <sup>d</sup>	evMSI <u>and</u> methylation tolerance above the validated laboratory's internal thresholds	evMSI <u>and</u> methylation tolerance within the range of negative controls
MMRDness testing by low-pass whole-genome sequencing/ LOGIC assay in blood leukocytes acc. To Chung et al. 2022 <sup>e</sup>	MMRDness score above the validated laboratory's internal thresholds	MMRDness score within the score range of negative controls

**Abbreviations:** MMR = mismatch repair; CMMRD – constitutional mismatch repair deficiency; MSI = microsatellite instability; acc. = according; PV = pathogenic variant; (L)PV = (likely) pathogenic variant.

<sup>#</sup>Validation cohort should include (i) at least eight CMMRD patients with different genotypes with respect to PVs and affected gene (for each of the four MMR genes at least one patient should be included), (ii) a large number of negative controls consisting of at least twenty adult individuals aged >40 years without cancer history and without a MMR gene (L)PV, (iii) at least ten confirmed MMR gene PV heterozygotes and, if available, (iv) *POLE* and *POLD1* PV heterozygotes.

<sup>a</sup>Ingham et al. Simple detection of germline microsatellite instability for diagnosis of constitutional mismatch repair cancer syndrome. *Hum Mutat* 2013; 34:847–52.

<sup>b</sup>Gallon et al. A sensitive and scalable microsatellite instability assay to diagnose constitutional mismatch repair deficiency by sequencing of peripheral blood leukocytes. *Hum Mutat* 2019; 40(5):649-655.

<sup>b</sup>Gallon et al. Constitutional microsatellite instability, genotype, and phenotype correlations in Constitutional Mismatch Repair Deficiency. *Gastroenterology* 2023; 164(4):579-592.

<sup>c</sup>González-Acosta et al. High-sensitivity microsatellite instability assessment for the detection of mismatch repair defects in normal tissue of biallelic germline mismatch repair mutation carriers. *J Med Genet* 2020; 57(4):269-273.

<sup>e</sup>Marín et al. A Validated Highly Sensitive Microsatellite Instability Assay Accurately Identifies Individuals Harboring Biallelic Germline PMS2 Pathogenic Variants in Constitutional Mismatch Repair Deficiency. *Clin Chem* 2024; 70(5):737–746.



<sup>d</sup>Bodo et al. Diagnosis of Constitutional Mismatch Repair-Deficiency Syndrome Based on Microsatellite instability and Lymphocyte Tolerance to Methylating Agents. *Gastroenterology* 2015; 149:1017–29.

<sup>e</sup>Chung et al. Genomic Microsatellite Signatures Identify Germline Mismatch Repair Deficiency and Risk of Cancer Onset. *J Clin Oncol* 2023; 41(4):766-777.

**Table 5: Criteria for the confirmation of CMMRD**

Genotype	MMR gene genetic testing reason					
Germline MMR gene variants identified (if two variants are identified, they must be confirmed to be in trans)	C4CMMRD criteria for cancer patient fulfilled (Rec.1)	Cancer <18 years with paediatric high TMB (Rec.2)	Cancer with MMR protein expression loss in neoplastic and non-neoplastic cells including tumour infiltrating lymphocytes and/or endothelial cells (Rec.3)	Cancer <18 years with heterozygous germline MMR gene (L)PV (Rec.4)	C4CMMRD criteria for children without cancer suspected to have NF1 /Legius syndrome and a negative <i>NF1/SPRED1</i> mutation analysis (Rec.6)	Incidental finding in WES or WGS performed for other reasons in a patient without cancer
PV/PV	√	√	√	√	√	√
PV/LPV	√	√(PPAP-)	√	√	√	√(AT+)
LPV/LPV	√	√(PPAP-)	√(AT+)	√(AT+)	√(AT+)	√(AT+)
(L)PV/VUS	√(AT+)	√(AT+)	√(AT+)	√(AT+)	√(AT+)	√(AT+)
VUS/VUS	√(AT+)	√(AT+)	√(AT+)	NA	√(AT+)	NA
(L)PV/X	√(AT+;mRNA+)	√(AT+;mRNA+)	√(AT+;mRNA+)	√(AT+;mRNA+)	√(AT+;mRNA+)	NA
VUS/X	√(AT+;mRNA+)	√(AT+;mRNA+)	√(AT+;mRNA+)	NA	√(AT+;mRNA+)	NA
X/X	√(AT+;mRNA+)	√(AT+;mRNA+)	√(AT+;mRNA+)	NA	√(AT+;mRNA+)	NA

**Abbreviations:** C4CMMRD = Care for CMMRD; MMR = mismatch repair; TMB = tumour mutation burden; PV = pathogenic variant; LPV = likely pathogenic variant; (L)PV = likely pathogenic or pathogenic variant; VUS = variant of unknown significance; WES = whole exome sequencing; WGS = whole genome sequencing; alleles are separated by / and X indicates one allele without an identifiable (L)PV or VUS.

NA = not applicable

√ = CMMRD confirmed without further ancillary test or transcript analysis

√(PPAP-) = CMMRD confirmed without further ancillary test if *POLE /POLD1* germline mutation excluded (i.e. polymerase proofreading associated polyposis negative: PPAP-)

√(AT+) = CMMRD confirmed if validated ancillary test positive for CMMRD (AT+)

√(AT+;mRNA+) = CMMRD confirmed if validated ancillary test positive for CMMRD(AT+) and evidence by transcript analysis for (a) faulty splicing (not explained by the identified variant) or (b) reduced expression of the wildtype allele(s) (mRNA+)

## 7.2 GENETIC COUNSELLING RECOMMENDATIONS

Recommendations		Strength
<b>Rec. 1</b>	Genetic counselling should be offered to parents and siblings of a confirmed CMMRD patient, preferentially by a multidisciplinary team with knowledge of CMMRD, consisting of a medical geneticist, a paediatric oncologist and a psychologist.	Strong
<b>Rec. 2</b>	To confirm their carrier status, parents of a CMMRD patient should be offered genetic testing for the (likely) pathogenic MMR gene variants found in their child.	Strong
<b>Rec. 3</b>	Cascade genetic testing for (likely) pathogenic variants should be offered to all adult relatives of a CMMRD patient, in both parental branches.	Strong
<b>Rec. 4</b>	Siblings of a genetically confirmed CMMRD patient should be offered genetic CMMRD testing regardless of age and phenotype.	Strong
<b>Rec. 5</b>	When performing CMMRD predictive testing in a minor or prenatal testing, pros and cons of revealing results of genetic testing regarding Lynch syndrome should be discussed on a case-by-case basis with the parents and the patient depending on their age.	Moderate
<b>Rec. 6</b>	If the diagnosis of CMMRD is not confirmed by the identification of two (likely) pathogenic variants in one MMR gene but by ancillary tests in the patient, siblings should probably be offered ancillary tests to exclude a CMMRD diagnosis for them.	Moderate

<b>Rec. 7</b>	Prenatal or preimplantation genetic testing should be discussed with parents of reproductive age of a CMMRD patient.	Strong
<b>Rec. 8</b>	Prenatal or preimplantation genetic testing should be discussed with couples of reproductive age if both carry a pathogenic variant in the same MMR gene.	Strong
<b>Rec. 9</b>	Testing the partner of a CMMRD patient for the MMR gene involved should probably be discussed during genetic counselling, considering possible consanguinity, common founder effect, and family history suggestive of Lynch syndrome.	Strong
<b>Rec. 10</b>	The partner of a Lynch syndrome carrier should be offered genetic testing of MMR genes if consanguinity is reported by the couple or the partner is coming from a population with a known founder variant or the family history of the partner is suggestive of Lynch syndrome and genetic testing has not been performed yet.	Strong
<b>Rec. 11</b>	The partner of a Lynch syndrome carrier should not be actively offered genetic testing of MMR genes in the absence of consanguinity, a known founder mutation or a family history suggestive of Lynch syndrome.	Moderate
<b>Rec. 12</b>	The child of a Lynch syndrome carrier should probably be offered CMMRD testing, if the child has clinical features that add up to $\geq 2$ C <sub>4</sub> CMMRD scoring points according to the revised criteria (Table 2: scoring points assigned to additional features in the patient).	Strong

### 7.3 SURVEILLANCE RECOMMENDATIONS

Recommendations	Strength
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<b>Rec. 1</b>	CMMRD patients and/or their parents should be educated about tumour risks associated with CMMRD.	Strong
<b>Rec. 2</b>	CMMRD patients and/or their parents should be educated about symptoms related to the main tumours, especially dyspnoea and superior vena cava syndrome for mediastinal lymphomas, symptoms associated with pancytopenia for leukaemia, neurological symptoms for brain tumours, and bleeding for colorectal tumours.	Strong
<b>Rec. 3</b>	Pros and cons should be discussed among the CMMRD patient and/or their parents and clinician to make a joint decision to participate in a surveillance program.	Strong
<b>Rec.4</b>	CMMRD patients and/or their parents should probably be encouraged to communicate their screening results in research projects or databases to improve knowledge on CMMRD.	Strong
<b>Rec. 5</b>	In children and adults with CMMRD, clinical examination should be performed every 6 months.	Strong
<b>Rec. 6</b>	Brain MRI should probably start at the initial CMMRD diagnosis or at least at the age of 2 years.	Strong
<b>Rec. 7</b>	In CMMRD patients up to age 20 years, brain MRI should be performed every 6 months.	Strong
<b>Rec. 8</b>	In CMMRD patients older than 20 years, a brain MRI should be performed at least annually.	Moderate
<b>Rec. 9</b>	The first brain MRI should probably be performed with contrast enhancement for all CMMRD patients.	Moderate

<b>Rec. 10</b>	In patients with CMMRD without a previous brain tumour, MRI should probably include anatomical sequence T2 FLAIR (if possible in 3D) combined with MRI diffusion sequence.	Moderate
<b>Rec.11</b>	In patients with CMMRD with a previous brain tumour, MRI should include anatomical sequences T2-FLAIR, diffusion sequence, and T1+ contrast enhancement if possible in 3D.	Moderate
<b>Rec. 12</b>	Abdominal ultrasound should probably not be performed to screen for abdominal lymphomas in CMMRD patients.	Weak
<b>Rec. 13</b>	Blood counts should probably not be performed to screen for haematological (pre-)malignancies in CMMRD patients.	Weak
<b>Rec. 14</b>	Colonoscopy should be performed at least annually in CMMRD patients and should probably start from the age of 6 years in children with CMMRD.	Strong
<b>Rec. 15</b>	Upper gastrointestinal endoscopy should be performed annually in CMMRD patients and should probably start at the same age as colonoscopy or at least at the age of 10 years.	Strong
<b>Rec. 16</b>	Upper endoscopy should probably use push enteroscopy and careful inspection of the ampullary region in CMMRD patients.	Moderate
<b>Rec. 17</b>	Upper endoscopy and colonoscopy should probably be done with coloration in the context of CMMRD.	Strong
<b>Rec. 18</b>	The frequency of upper or lower endoscopy should probably increase up to 6 months-interval once polyps are detected in the context of CMMRD.	Strong
<b>Rec. 19</b>	Digestive tract surveillance for CMMRD patients, including children, should probably be done in a centre with gastroenterologists experienced in Lynch syndrome screening.	Moderate

<b>Rec. 20</b>	The interval between two digestive tract examinations should not exceed 12 months for CMMRD patients.	Strong
<b>Rec. 21</b>	Video capsule endoscopy should be performed annually in CMMRD patients and should probably be performed from the age of 10 years.	Strong
<b>Rec. 22</b>	Gynaecologic surveillance should probably be performed annually from age 20 years in CMMRD patients and should include clinical examination and transvaginal ultrasound.	Strong
<b>Rec. 23</b>	Prophylactic hysterectomy should probably be discussed once family planning of the CMMRD patient is completed.	Moderate
<b>Rec. 24</b>	Annual urine cytology and urine dipstick should probably not be offered to CMMRD patients.	Moderate
<b>Rec. 25</b>	Abdominopelvic ultrasound for gynaecological and urinary tract cancer screening should probably be offered annually to CMMRD patients, starting at 20 years of age.	Strong
<b>Rec. 26</b>	Breast cancer screening should probably follow general population guidelines for CMMRD patients.	Moderate
<b>Rec. 27</b>	Whole body MRI should probably be offered to CMMRD patients at least once, at diagnosis or when anaesthesia is no longer required, for a general screening of low-grade tumours and malformations to guide targeted screening.	Strong
<b>Rec. 28</b>	Resection or specific surveillance of low-grade lesions should be offered to CMMRD patients.	Strong
<b>Rec. 29</b>	Even though evidence of its efficacy in screening is still weak in CMMRD, whole-body MRI should probably be discussed with CMMRD patients as an option for annual surveillance.	Moderate

## 7.4 QUALITY OF LIFE RECOMMENDATIONS

Recommendations		Strength
<b>Rec. 1</b>	Psychological support should be offered to the patient and the family during the entire process of evaluation before the diagnosis of CMMRD.	Strong
<b>Rec. 2</b>	Psychological support should be offered to patients with CMMRD and their families at any time during treatment and cancer surveillance.	Strong
<b>Rec. 3</b>	Age adapted education about CMMRD should probably be offered to CMMRD patients and their families.	Strong
<b>Rec. 4</b>	Healthcare professionals involved in diagnosis and surveillance should address the psychosocial implications of a diagnosis of CMMRD.	Strong

## 7.5 CLINICAL MANAGEMENT RECOMMENDATIONS

Recommendations		Strength
<b>Rec. 1</b>	Multiple patients with CMMRD have been cured from a cancer diagnosis. Thus, in a CMMRD patient diagnosed with cancer, a curative approach should be considered and evaluated.	Strong
<b>Rec. 2</b>	For several cancer types, no CMMRD specific treatment recommendations exist. Treatment of patients with CMMRD related neoplasms should, therefore, probably be discussed in a multidisciplinary board with a treating physician, an expert for the patient's cancer type as well as a CMMRD expert.	Strong

<b>Rec. 3</b>	Patients with CMMRD associated neoplasms should probably be included in clinical trials whenever possible.	Strong
<b>Rec. 4</b>	CMMRD is probably not a contraindication for radiotherapy, if indicated.	Moderate
<b>Rec. 5</b>	CMMRD is probably not a contraindication for haematopoietic stem cell transplantation, if indicated.	Moderate
<b>Rec. 6</b>	Temozolomide should probably be avoided in patients with CMMRD-associated high-grade glioma.	Strong
<b>Rec. 7</b>	The use of immunotherapy with a PD1 inhibitor should be considered for CMMRD patients with high-grade glioma, preferentially within a clinical trial.	Strong
<b>Rec. 8</b>	CMMRD-associated low grade glioma should probably be resected whenever possible without excessive neurological risks.	Strong
<b>Rec. 9</b>	Front-line treatment of CMMRD-associated medulloblastoma should probably not differ from treatment of sporadic medulloblastoma/primitive neuro-ectodermal tumours.	Moderate
<b>Rec. 10</b>	In case of CMMRD-associated non-Hodgkin lymphoma, chemotherapy should probably be similar to the treatment of the same tumour without CMMRD.	Moderate
<b>Rec. 11</b>	In case of a second primary non-Hodgkin lymphoma in a CMMRD patient, standard first-line treatment adapted to the non-Hodgkin lymphoma subtype taking into account cumulative doses of chemotherapy previously received should probably be given rather than a relapse treatment.	Moderate



<b>Rec. 12</b>	In case of CMMRD-associated leukaemia, chemotherapy should probably be similar to the treatment of the same cancer without CMMRD.	Moderate
<b>Rec. 13</b>	In case of diagnosis of a cancer of the Lynch spectrum in a CMMRD patient, treatment guidelines designed for patients with Lynch syndrome associated tumours should be followed.	Strong
<b>Rec. 14</b>	Immunotherapy should be recommended as front-line treatment of large, unresectable or metastatic colorectal tumours in a CMMRD patient	Strong
<b>Rec. 15</b>	Immunotherapy should be performed front-line for all extra-colorectal Lynch-related tumours in CMMRD patients ideally in therapeutic trials.	Strong
<b>Rec. 16</b>	Immunotherapy should be discussed and encouraged within an expert centre for any non-Lynch related tumour at any time during treatment (diagnosis or relapse) of a CMMRD patient, especially if standard therapeutic guidelines offer only low chance of cure.	Moderate
<b>Rec. 17</b>	CMMRD patients with multiple colonic adenomas should probably be surgically managed according to guidelines developed for other polyposis syndromes.	Strong
<b>Rec. 18</b>	CMMRD patients may present with multiple tumours at the same time or may develop additional tumours during treatment. Thus, cancer surveillance around the time of diagnosis and during the period of cancer treatment should be offered.	Strong
<b>Rec. 19</b>	In CMMRD patients with a suspected relapse, a second primary disease should be considered. This may influence the treatment choice.	Strong

<b>Rec. 20</b>	In case of relapse of a CMMRD-associated tumour, molecular analysis of samples at initial diagnosis and relapse should be performed to differentiate a relapse from a second primary tumour.	Strong
<b>Rec. 21</b>	Fresh tumour specimens should be collected and stored (or directly molecularly analysed) whenever possible and if the CMMRD patient and/or their family approves. This may be relevant for research as well as for clinical purposes (e.g. see Rec 19).	Strong
<b>Rec. 22</b>	Advantages and potential side effects of preventive treatment with acetylsalicylic acid should probably be discussed with CMMRD patients.	Moderate
<b>Rec. 23</b>	CMMRD patients with IgG/A reduced levels/deficiency should not be treated to compensate for the inherent deficit in the absence of clinical manifestations.	Strong

## 8. METHODS FOR GUIDELINE DEVELOPMENT

### 8.1 FORMULATING AND GRADING STATEMENTS AND CONSENSUS BUILDING

#### Literature search

The systematic literature search was performed by Agència de Qualitat i Avaluació Sanitàries de Catalunya (AQuAS) in 3 databases (Pubmed, Cinhal and Embase) and retrieved 332 unique references using the following terms: CMMRD [Title/Abstract] OR CMMR-D [Title/Abstract] OR "constitutional mismatch repair deficiency" [Title/Abstract] OR "constitutive mismatch repair deficiency" [Title/Abstract] OR "biallelic mismatch repair deficiency" [Title/Abstract] OR bMMRD [Title/Abstract] OR "mismatch repair cancer syndrome" [Title/Abstract] OR "OMIM 276300". Another 20 articles were selected using citation searching. Out of the 352 references screened, 258 were excluded because of a wrong design (conference abstracts), wrong language (not English), wrong outcome (not responding to any of the questions of

interest), wrong population (not CMMRD), and/or because the presented data was already included from other references. The remaining 94 articles were summarised by AQuAS in a comprehensive literature review. The core working group drafted the recommendations for this guideline by building on this comprehensive literature review with additional articles they identified and their expert knowledge.

### **Method for formulating recommendations**

In day-to-day practice, clinicians will not have the time to explore the evidence as thoroughly as a Guideline Group, nor devote as much thought to the trade-offs, or the possible underlying values and preferences in the population. Therefore, the Core Working Group has made recommendations even when confidence in effect is low, but a recommendation is desirable due to potential patient benefit, with (potential) negative consequences being considered. Such recommendations have been classified as 'weak'. The recommendations have been graded on the quality of evidence; balance between benefits and harms; include the values and preferences of patients; and consider the feasibility, equity & acceptability of implementation and use.

Literature was reviewed along with expert opinion to draft recommendations based on literature and experts' experiences and knowledge.

Recommendations were written in one of four stylistic formats: Should, Should Probably, Should Probably Not, Should Not:

- Should & Should Not, were taken to mean - most well-informed people (those who have considered the evidence) would take this action.
- Should Probably & Should Probably Not, were taken to mean - the majority of informed people would take this action, but a substantial minority would not.

### **Grading of the recommendations**

As the volume of peer-reviewed evidence for rare diseases is typically limited due to the small population sizes, and it is unlikely that the evidence will ever reach a fraction of that for a more common disease, it creates a difficulty when considering the grading of the strength of

evidence using Grading of Recommendations Assessment, Development and Evaluation (GRADE).

As is typical for many rare diseases, the volume of peer-reviewed evidence available to consider for these guidelines was small and came from a limited number of articles, which typically reported on small samples or series. If the evidence is categorised and then graded using standard approaches, that are designed to differentiate evidence, in circumstances when there are large numbers of papers and there are likely to be more trials, then its small volume means it would be graded as low. This is not an accurate reflection of the combination of the experts' experience and clinical consensus with the available evidence. This is further compounded as there is a low likelihood of additional volumes of evidence that could change the recommendation.

For this reason, and to balance the weight of both published evidence and quantify the wealth of expert experience and knowledge, ERN GENTURIS uses the following scale to grade the recommendation:

<b>Strength</b>	<b>Grading of Recommendation</b>
Strong	Expert consensus AND consistent evidence
Moderate	Expert consensus WITH inconsistent evidence AND/OR new evidence likely to support the recommendation
Weak	Expert majority decision WITHOUT consistent evidence

Expert consensus (an opinion or position reached by a group as whole) or expert majority decision (an opinion or position reached by the majority of the group) was established after reviewing the results of the modified Delphi approach within the Core Working Group.

The findings of the literature review were organised against the PICO questions and outcomes.

In addition, strength of recommendation has been determined through a consensus-based approach (modified Delphi) and through active engagement of affected individuals and

parent representatives, specifically balancing the desirable and undesirable consequences of surveillance and alternative care strategies, quality of evidence, and values and preferences held by the patient representatives.

The quantification of strength for a recommendation is a composite of harm and benefit. As a general note for these recommendations, the harms a recommendation seeks to address are often clear, however the magnitude of the benefit of a specific recommendation are often not as clear. Therefore, the published evidence for a recommendation can often be classified 'weak', even when experts are convinced that the recommendation is correct.

### **Consensus building using a modified Delphi approach**

After drafting recommendations amongst the Guideline Group these were subjected to a modified Delphi assessment. Delphi is a structured communication technique or method in which opinions of a large number of experts are asked on a topic in which there is no consensus, and this was used as a consensus building exercise. The goal is to reach consensus after several rounds of questionnaires.

Experts included in this exercise were the eight members of the Core Working Group (including one patient representative), the CMMRD Guideline Group (n=11), as well as other (external) experts identified by the Guideline Group (n=53).

The survey consisted of two rounds, in which the threshold for consensus was defined by a simple majority of the survey participants agreeing with the recommendation (>60% rated "agree" or "totally agree"). Recommendations were graded using a 4-point Likert scale (totally disagree, disagree, agree, totally agree) and a justification for the given rating was obligatory. Even if consensus was met, recommendations were still modified if a higher consensus was thought achievable from written responses.

All recommendations formulated by the Guideline Group (Chapter 7) were subjected to the Delphi procedure. The facilitator of the Delphi survey provided anonymised summaries of the experts' decisions after each round as well as the reasons they provided for their judgements. After two Delphi rounds, an agreement of 68% to 100% (median 92) was reached for all 82 recommendations and their strength was graded as weak (n=2), moderate

(n=23) or strong (n=57). The high rate of weak and moderate evidence is mainly due to the paucity of data in the literature.

We would like to thank the 53 experts that were specifically consulted to participate in the Delphi survey:

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## 8.2 INTERNAL AND EXTERNAL REVIEW

ERN GENTURIS has actively involved external experts from different speciality areas that are relevant to the scope of the guideline to review the findings and recommendations developed in this guideline by participation in the Guideline Group or as a Delphi participant.

In addition, the CMMRD Guideline Group engaged with the European Journal of Human Genetics as an independent review of the guideline.

ERN GENTURIS first published the Guideline for the diagnosis, counselling, surveillance, quality of life, and clinical management of CMMRD on 17 October 2024.

## 8.3 TIMELINE AND PROCEDURE FOR UPDATING THE GUIDELINE

Any new evidence that has been published will be updated to the Network clinical leads on an annual basis and consideration for updating the guideline thereafter. New versions will be published on the Network's website and circulated through the ERN GENTURIS Members.

## 8.4 FUNDING AND FINANCIAL SUPPORT

This guideline has been supported by the European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS). ERN GENTURIS is funded by the European Union. For more information about the ERNs and the EU health strategy, please visit <http://ec.europa.eu/health/ern>. Potential conflict of interest for the individual authors and Delphi participants are listed in chapter 5.

## 9. SUMMARY OF EVIDENCE AND RECOMMENDATIONS

### 9.1 DIAGNOSIS - SUMMARY OF EVIDENCE AND GUIDELINE RECOMMENDATIONS

In these guidelines, we provide recommendations as to when CMMRD testing should be offered to a patient with or without malignancy, recommendations for a testing strategy that facilitates a definite diagnosis in a suspected patient, and recommendations as to when CMMRD is confirmed or refuted in a suspected patient. The evidences for these guideline recommendations are discussed in the following summary.

#### Indication criteria

Several different scenarios as to when CMMRD testing should be offered have been described and guidelines have been developed for these scenarios. These were evaluated to develop the recommendations given here.

The first indication criteria for CMMRD testing were developed for cancer patients by the European C<sub>4</sub>CMMRD consortium (Wimmer et al., 2014). These “C<sub>4</sub>CMMRD criteria” use a system of scoring points based on the clinical phenotype of the patient and are likely to be highly sensitive as shown by subsequent studies (Baris et al., 2016; Lavoine et al., 2015). The scoring system assigns a different number of points to different tumour types depending on their representation within the malignancy spectrum of CMMRD. Specifically, for each tumour type, the level of association with CMMRD was estimated by comparing the proportion of cancer diagnoses of that tumour type in CMMRD to the proportion of cancer diagnoses of that tumour type in the general population of an equivalent age. Tumour types with a strong association with CMMRD were assigned three points. Patients with these tumour types should be offered CMMRD testing regardless of whether they show additional (non-neoplastic) features of CMMRD. Malignancies that are overrepresented in CMMRD but less specific were assigned two points. CMMRD testing should be offered in patients with these malignancies only when additional features or tumours that increase the total score to at least three points are present. All other malignancies were assigned one point. Hence, additional tumours or features strongly suggestive of CMMRD that increase the total score to at least three C<sub>4</sub>CMMRD scoring points need to be present in a patient with one or more of these malignancies to indicate CMMRD testing. Similar to the tumour types, several non-

malignant features indicating CMMRD were weighted with one point or two points according to their specificity for CMMRD based on their frequency in the general population.

Multiple subsequent studies have confirmed the overrepresentation of cancers assigned three or two points in the "C<sub>4</sub>CMMRD criteria" in CMMRD patients (Amayiri et al., 2016; Baris et al., 2016; Durno et al., 2017a; Guerrini-Rousseau et al., 2019; Khdair-Ahmad et al., 2021; Lavoine et al., 2015). However, updates in terminology and our knowledge of the CMMRD phenotype require these guidelines to be revised.

Two tumours which were assigned two points in the original "C<sub>4</sub>CMMRD criteria", non-Hodgkin lymphoma (NHL) of the T-cell lineage and supra-tentorial primitive neuroectodermal tumours (sPNET), should now be differently named. CMMRD-associated NHL of the T-cell lineage falls mainly in the entity of T-cell lymphoblastic lymphoma (T-LBL). sPNET no longer exists as a specific tumour entity and CMMRD-associated brain tumours previously classified as sPNET fall mainly into the group of diffuse HGGs. Therefore, the "C<sub>4</sub>CMMRD criteria" were, here, revised accordingly.

Equally, new findings regarding non-malignant features of CMMRD necessitate the revision of the original "C<sub>4</sub>CMMRD criteria". Specifically, multiple developmental venous anomalies (DVAs, also known as cerebral venous angiomas) of the brain were identified as a feature that is very frequent in CMMRD (73-100% patients) (Guerrini-Rousseau et al., 2019; Kerpel et al., 2020; Raveneau et al., 2024; Shiran et al., 2018), but is rare (<1% of individuals) in the general population (Gokce et al., 2014). Hence, this new feature is now included with two points in the revised C<sub>4</sub>CMMRD criteria. Note however, that a single DVA was found in 10% of NF1 patients analysed, most of whom had either low-grade or high-grade gliomas, in 14% of patients with Lynch syndrome and high-grade gliomas, and in 6% of all sporadic patients with high-grade gliomas, while multiple DVA were found in 83% of CMMRD with high-grade gliomas and 3% of the analysed NF1 patients, but in none of the sporadic or Lynch syndrome patients with HGGs (Raveneau et al., 2024).

Paediatric systemic lupus erythematosus, which is rare in the general population with a prevalence of 3.3-3.8 per 100,000 children, was found in a total five of roughly 200 reported CMMRD patients (2.5%) and is, hence, also significantly overrepresented in CMMRD (Toledano et al., 2020; Vasantachart et al., 2022). Paediatric systemic lupus erythematosus was, therefore, also included with one point in the revised C<sub>4</sub>CMMRD criteria.

So far, unpublished results of the Gustave Roussy university hospital, which systematically applied the original C<sub>4</sub>CMMRD criteria, showed that assigning two points to two or more hyperpigmented and/or hypopigmented skin alterations was too inclusive leading to testing for CMMRD in many non-CMMRD patients. This caused often unnecessary anxiety in patients and their families as well as extensive diagnostic effort to refute the diagnosis. Therefore, this feature was assigned only one point in the revised criteria. However, clinical signs of neurofibromatosis type 1 (NF1) and/or  $\geq 4$  hyperpigmented and/or hypopigmented skin alterations with a diameter over 1 cm remained weighted with two points. Note, that it has been shown in a small number of patients with CMMRD that an NF1 phenotype may result from a (postzygotic) *NF1* pathogenic variant (PV) identifiable in blood leukocytes (Alotaibi et al., 2008; Guerrini-Rousseau et al., 2024). Therefore, it is advisable to consider CMMRD also in genetically confirmed (mosaic) NF1 patients who have a malignancy that is not typical of NF1, such as a paediatric diffuse HGG or T-cell lymphoblastic lymphoma. Finally, for clarity of presentation, all non-malignant features were divided into features present in the patient and those present in the family. The revised "C<sub>4</sub>CMMRD criteria" (Rec. 1) are summarised in Table 2.

In addition to clinical features, CMMRD may be suspected from evaluation of molecular features of the patient and/or their tumour.

Tumour profiling by whole exome or genome sequencing (WES, WGS) of paediatric cancers is increasingly performed/available, either as part of the clinical work-up of these tumours or in a research setting. WES and WGS (and sometimes gene panel sequencing, typically covering  $\geq 1$  megabase of DNA sequence) can be used to estimate tumour mutational burden (TMB). TMB is quantified by the number of somatic mutations per megabase (Mut/Mb) of DNA sequence covered. TMB can have implications for prognosis and therapy with tumours separated into two groups, those with a high TMB (also called hypermutated) and those with a low TMB. This dichotomisation generally uses a threshold of 10 Mut/Mb. However, sequencing and analysis methods vary and so does the classification threshold used (Sha et al., 2020). Paediatric tumours are generally characterised by a low TMB (Gröbner et al., 2018). In contrast, Shlien et al. showed that nearly all CMMRD brain and less frequently other CMMRD-associated tumours are ultra-hypermutated (generally defined as a TMB >100

Mut/Mb). Ultra-hypermutation has been linked to concurrent MMR deficiency and polymerase epsilon or delta proofreading deficiency. In CMMRD, the latter is acquired by early somatic PVs in *POLE* or *POLD1* (Shlien et al., 2015). Further studies confirmed that (ultra-)hypermutation is a common feature in different types of CMMRD-associated tumours (Bouffet et al., 2016; Campbell et al., 2017; Oshrine et al., 2019). MMR deficiency and concurrent MMR and polymerase proofreading deficiency are associated with specific COSMIC signatures such as [SBS6](#), [SBS14](#), [SBS15](#), [SBS20](#), [SBS21](#), [SBS26](#) or [SBS44](#) and these signatures are found in hypermutated tumours of patients with CMMRD (Hodel et al., 2020). Although these features are highly indicative for CMMRD, they are not pathognomonic as paediatric hypermutated tumours may also arise in patients with specific germline *POLE* PV (Sehested et al., 2022) or in patients with heterozygous *POLD1* or *POLE* germline PVs combined with a heterozygous *PMS2* PV (Berrino et al., 2022; Michaeli et al., 2022; Schamschula et al., 2022). Taken together, a TMB that is unusually high for a paediatric tumour should raise suspicion of CMMRD and even more so if the mutational signature indicates MMR deficiency with or without concurrent polymerase proofreading deficiency (Rec. 2).

Biallelic truncating and other MMR gene PVs typically lead to expression loss of the respective MMR protein, identifiable by immunohistochemical staining (IHC). Loss of expression of MSH2 and MLH1 often also leads to loss of their respective heterodimerisation partners MSH6 and PMS2 (Mojtahed et al., 2011). Universal screening by IHC of all colorectal and other Lynch syndrome-associated tumours is recommended by many national and international medical professional organizations and is performed in many European countries (Balmana et al., 2013; Stoffel et al., 2015). In contrast to LS, where MMR protein expression loss is restricted to neoplastic cells, CMMRD is often characterized by MMR protein expression loss in both neoplastic and non-neoplastic cells including endothelial cells in intratumoural blood vessels and tumour infiltrating leukocytes (Bakry et al., 2014), observation of which should entail CMMRD diagnostic work-up (Rec. 3).

Single and trio germline WES and WGS is performed increasingly in paediatric cancer patients in a clinical or research setting and may reveal a heterozygous (L)PV in one of the MMR genes (Kratz et al., 2022). It is well known that PV in the notoriously difficult to analyse *PMS2*, but also in the other MMR genes, may escape detection by WES or WGS. Therefore,

identification of a heterozygous (L)PV in *PMS2* or in one of the other three MMR genes in a paediatric, adolescent or young adult cancer patient should entail CMMRD diagnostic work-up (Rec. 4).

To potentially strengthen the suspicion of CMMRD in a patient who fulfils one of the three molecular-based criteria for CMMRD testing outlined above (Rec. 2-4), a careful family history assessment and physical examination of the patient should be performed to determine if the C<sub>4</sub>CMMRD criteria for cancer patients are also fulfilled (Rec. 5). Note that in Rec. 12 and Table 5 the criteria for confirmation of CMMRD slightly differ between patients who only fulfil the indication for testing according to Rec. 2-4 when compared to patients who also fulfil the indication outlined in Rec. 1. A systematic recording of the identified neoplastic and non-neoplastic clinical findings and their family history will help to improve our knowledge on the phenotypic presentation of CMMRD.

CMMRD phenotypically overlaps with neurofibromatosis type 1 (NF1) and Legius syndrome (LGSS). CALMs are present in 62–97% of CMMRD patients, and approximately 20% of CMMRD patients show more than one diagnostic NF1 feature (Legius et al., 2021; Wimmer et al., 2017b). Hence, prior to malignancy, CMMRD may be indistinguishable from NF1/LGSS, as exemplified by several cases who received an incorrect initial diagnosis of NF1 – for example, see (Suerink et al., 2018). CMMRD is therefore a possible albeit rare differential diagnosis in otherwise healthy children with CALMs (with or without other clinical signs of NF1/LGSS) when no causative *NF1* or *SPRED1* PV is identified, and no signs of NF1 are found in the parents. Although mosaic/segmental NF1 is the most likely differential diagnosis in a child with CALMs when no causative germline variants are identified in *NF1* or *SPRED1*, NF1 somatic testing can be omitted prior to CMMRD testing since it requires invasive procedures (Suerink et al., 2019a). Testing of these children for CMMRD may provide an opportunity for CMMRD cancer surveillance prior to onset of the first malignancy. However, it was estimated and later experimentally confirmed that the prevalence of CMMRD is as low as 0.4% in suspected sporadic NF1 children from non-consanguineous parents for whom no constitutional/germline *NF1* or *SPRED1* PV is identified (Perez-Valencia et al., 2020; Suerink et al., 2019a). Considering this low prevalence and balancing the benefits of a diagnosis in these children against the potential harms of CMMRD testing in a healthy child, the

C<sub>4</sub>CMMRD consortium has formulated consensus guidelines advocating testing of CMMRD in preselected patients with a higher a priori risk, rather than reflex testing of all suspected sporadic NF1/LGSS children lacking a causative *NF1* or *SPRED1* variant (Suerink et al., 2019a). These C<sub>4</sub>CMMRD consensus guidelines propose criteria for preselection, including exclusion of NF1/LGSS with highly sensitive mutation analysis (including methods that identify and characterise unusual splice variants) and, in addition to the features that led to the suspected diagnosis of NF1/LGSS, presence of features in the child and/or their family that are indicative for CMMRD (see Table 3; (Suerink et al., 2019a)). However, the sensitivity and specificity as well as positive predictive value and negative predictive value of these criteria are currently unknown. Therefore, CMMRD testing should probably be offered in specialised centres, following an interdisciplinary discussion, to all children who fulfil the preselection criteria (Rec. 6, Table 3).

### **Testing strategy**

Diagnosing CMMRD in a patient has important management implications for the patient and their entire family. Furthermore, raising the suspicion of CMMRD in a child is likely to cause anxiety in the parents and the child. Hence, it should be the aim of any testing strategy to come to a definite diagnosis that either confirms or refutes CMMRD in the patient, and to identify the causative variants in the relevant MMR gene (Rec. 7), so that subsequent cascade testing of relatives will be possible (see also chapter 9.2. Summary of evidence and guideline Recommendations for Genetic Counselling).

The first diagnostic steps in patients with suspected CMMRD can follow the protocols developed for Lynch syndrome, which includes IHC of the four MMR proteins to assess loss of the affected MMR protein (Rec. 8). Especially if tumour or biopsy tissue of solid tumours is available, IHC can be effectively employed and is easily accessible at most clinical pathology laboratories. It is a comparably inexpensive test with a low turnaround time (Southey et al., 2005). Performing it prior to genetic testing will have the advantage that it may guide targeted gene mutation analysis and may reinforce the suspected diagnosis. Depending on the nature of the causative MMR gene variants, tumours of CMMRD patients often show nuclear expression loss of the affected MMR protein in neoplastic and in non-neoplastic cells



including intratumoural endothelial cells and tumour-infiltrating leukocytes (see also Rec. 3). This is highly indicative for CMMRD, but it should be kept in mind that especially, but not only, PMS2 is expressed in some tissues at very low levels which may lead to an apparently negative staining in all cells of a tissue that is not related to CMMRD. Due to this potential pitfall, IHC should be used to confirm the diagnosis CMMRD only in suspected CMMRD patients for whom no germline DNA/RNA is available to confirm the diagnosis by the criteria outlined in recommendation 12 and Table 5 (see Rec.14). Furthermore, a normal IHC staining result cannot exclude CMMRD, because non-truncating variants causative of CMMRD may be associated with expression of the non-functional but immunohistochemically stainable protein.

Over 50% of the CMMRD cases are caused by biallelic variants in *PMS2*. Due to the presence of pseudogenes, specifically *PMS2CL* which shares homologous sequences with the 3' region of *PMS2* (exons 9 and 11-15), analysis of this gene by standard sequencing techniques (Sanger and next generation sequencing (NGS)) can be challenging. Regions of *PMS2* are even considered a "sequencing dead zone" (Mandelker et al., 2016). Several strategies have been developed to effectively identify (L)PV variants in the functional *PMS2* gene (Etzler et al., 2008; van der Klift et al., 2016; Vaughn et al., 2011; Vaughn et al., 2010; Wernstedt et al., 2012). These or other strategies that effectively circumvent potential pitfalls of *PMS2* mutation analysis should be available in laboratories offering CMMRD testing to assure that *PMS2* variants in CMMRD are reliably and effectively detected (Rec. 9).

Transcript analysis can identify potential splice effects of a variant, which may be important for its classification. Transcript analysis is also a very useful tool to assess expression of the alleles, which can indicate presence of variants that escape detection by targeted (e.g. gene panel or exome) sequencing of genomic DNA (gDNA), such as deep-intronic variants. Hence, transcript analysis may become necessary to come to a definitive diagnosis that either confirms or excludes CMMRD in cases where gDNA sequencing cannot render an unequivocal result (Rec. 9), e.g. in cases where variants of unknown significance (VUS) or only one or no MMR gene variants are identified (for further details see below the summary of evidences for diagnostic criteria recommendations).

Due to the limitations inherent to genetic testing, ancillary tests to assess the underlying molecular pathology of CMMRD, that is the constitutional loss of MMR function, have been developed and used to confirm CMMRD in cases where genetic testing renders an inconclusive result (e.g. by detection of one or more VUS or failure to detect biallelic PVs). Table 4 lists available ancillary tests that mostly assess microsatellite instability (MSI) in constitutional DNA extracted from blood leukocytes, which is a hallmark (arguably pathognomonic) feature of CMMRD. These assays include germline MSI (gMSI) which is based on fragment length analysis of three MSI markers (Ingham et al., 2013). This assay has limitations and is insensitive to *MSH6*-associated CMMRD because it relies on dinucleotide repeat MSI markers. NGS-based assays that test for constitutional MSI in larger panels of selected markers have been shown to have 100% sensitivity and specificity (Gallon et al., 2019; Gallon et al., 2023; Gonzalez-Acosta et al., 2020). Equally, MSI analysis of Epstein Barr Virus-immortalised lymphocytes using fragment length analysis of mononucleotide repeat MSI markers and parallel analysis of cell tolerance to methylating agents, another functional consequence of CMMRD, can both confirm and largely exclude a CMMRD diagnosis (Bodo et al., 2015). These assays have all been evaluated on blinded cohorts of patients and control samples collected by the European C4CMMRD consortium. The “low coverage whole genome instability characterization” (LOGIC) assay can also confirm CMMRD through low-pass whole-genome sequencing and assessment of genome-wide MSI (MMRDness). This assay has been developed and evaluated by the International Replication Repair Deficiency Consortium (IRRDC) (Chung et al., 2021; Chung et al., 2023).

These assays are currently available only in a few European laboratories and some have not been clinically validated and are available only as a research test. None of the constitutional MSI assays is currently available as a commercial kit. Hence, it is unlikely that all laboratories performing genetic CMMRD testing will establish one of these assays. Given the rarity of CMMRD, it is reasonable to recommend that laboratories offering CMMRD testing within Europe should have agreements with a laboratory offering one or more of these assays to assure an ancillary test is available to complement genetic testing if needed to reach a definitive diagnosis (Rec. 10). When testing for CMMRD as differential diagnosis of NF1 in children without malignancy, a definitive conclusion regarding presence or absence of CMMRD should be reached. Therefore, testing in this situation should be performed only in

specialised centres (Suerink et al., 2019a). In these centres, one or more validated ancillary assays should be available, either in their own laboratory or through cooperation partners (Rec. 11).

### **Diagnostic criteria**

Identification of two variants in one of the MMR genes classified as (L)PV, which are confirmed to be located in trans by genetic testing, confirms the diagnosis CMMRD in a patient who fulfils one or more of the suggested criteria for CMMRD testing (see above as well as Rec.1, Rec. 2, Rec.3, Rec. 4 and Rec.6). Note that a careful family history assessment and physical examination should be performed for all patients who fulfil the CMMRD testing criteria as described in Rec. 2, Rec.3, or Rec. 4 (Rec. 5).

In cases where a homozygous or two compound heterozygous LPV are identified and the patient neither fulfils the C<sub>4</sub>CMMRD criteria for cancer patients (Rec.1) nor has a hyper- or ultra-mutated tumour (Rec. 2) the level of CMMRD suspicion is lowered and CMMRD should be considered definite only when an ancillary test confirms the diagnosis. The same should be the case when an LPV in trans with a PV is incidentally identified by WES/WGS performed for other reasons in a patient without cancer.

Confirmation of CMMRD by an ancillary test is also needed if genetic testing in one of the MMR genes identifies one or two variants which are in trans but cannot both be classified as (L)PV, according to the most recent internationally accepted criteria (by the [ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel](#); for criteria specifications, see <https://cspec.genome.network/cspec/ui/svi/affiliation/50099>).

Due to its inherent limitations even when combining different methods including transcript analysis, genetic testing may fail to identify a causative MMR gene alteration. However, even in such cases, application of appropriately designed transcript analyses should be able to confirm expression loss of the “apparent wild-type” allele(s) in a CMMRD patient. Therefore, loss of allele expression or faulty splicing due to an unidentified splice variant should be confirmed in addition to performing a validated ancillary test to confirm the diagnosis of CMMRD in a suspected patient in whom only one (L)PV or VUS or no potentially causative MMR gene variant is identified.

Showing expression of two alleles at least one being a full-length wild-type transcript largely refutes CMMRD as does the exclusion by ancillary testing in a patient without two (L)PV in trans. Note that gMSI testing cannot be used to exclude MSH6-associated CMMRD.

When applying the criteria summarized in Rec.12 and Table 5 to confirm a suspected CMMRD diagnosis, physicians should use their medical expertise to judge the plausibility of the combined genetic and ancillary testing result and question these results if unexplainable discrepancies become apparent. An interdisciplinary team (geneticist, physician, pathologist) would be necessary to discuss these difficult cases.

Specific heterozygous *POLE* germline PV as well as a digenic combination of a heterozygous germline *POLE* or *POLD1* PV and a heterozygous germline MMR gene PV have been shown to cause a phenotype reminiscent of CMMRD (Berrino et al., 2022; Lindsay et al., 2019; Michaeli et al., 2022; Schamschula et al., 2022; Sehested et al., 2022; Wimmer et al., 2017a). Therefore, cancer patients with or without heterozygous MMR (L)PV or VUS in whom the suspected diagnosis of CMMRD cannot be confirmed should probably be tested for germline *POLE* and *POLD1* exonuclease domain variants (Rec. 13). At least in cases with a paediatric high TMB, this should be done in parallel with genetic testing of the MMR genes.

Recommendations		Strength
<b>Rec. 1</b>	CMMRD testing should be offered to all cancer patients who reach a minimum of three scoring points according to the revised C <sub>4</sub> CMMRD indication criteria (Table 2).	Strong
<b>Rec. 2</b>	CMMRD testing should be offered to all cancer patients aged <18 years with a tumour that has a paediatric-high* tumour mutational burden (TMB), regardless of presence or absence of a somatic <i>POLE</i> or <i>POLD1</i> pathogenic variant.  *(Gröbner et al., 2018; Merino et al., 2020)	Strong
<b>Rec. 3</b>	CMMRD testing should be offered to all cancer patients with a tumour that has expression loss of one or more of the four MMR	Strong

	proteins by immunohistochemical staining in neoplastic and in non-neoplastic cells including tumour infiltrating leukocytes and/or endothelial cells.	
<b>Rec. 4</b>	CMMRD testing should be offered to all cancer patients aged <18 years in whom a heterozygous (likely) pathogenic variant in one of the MMR genes was found by germline sequencing.	Strong
<b>Rec. 5</b>	A family history assessment and physical examination should be performed for any patient who fulfils inclusion criteria of CMMRD testing as described in Rec. 2-4.	Strong
<b>Rec. 6</b>	CMMRD testing should probably be offered in expert centres following an interdisciplinary discussion to all children suspected to have sporadic NF1/Legius syndrome without cancer and without an <i>NF1/SPRED1</i> germline (L)PV after comprehensive genetic analysis and who have at least one additional feature defined by the C4CMMRD guidelines (Suerink et al 2018, Table 3).	Strong
	<b>Testing strategy</b>	
<b>Rec. 7</b>	Any testing strategy should aim to come to a definite diagnosis that either confirms or refutes CMMRD in the patient, and to identify the causative variants in the relevant MMR gene.	Strong
<b>Rec. 8</b>	Wherever possible, CMMRD testing of a patient with a (pre-)malignancy should include immunohistochemical staining of all four MMR proteins in tumour tissue to determine MMR protein expression in neoplastic and in non-neoplastic cells, including tumour infiltrating leukocytes and/or endothelial cells.	Strong
<b>Rec. 9</b>	The laboratory performing genetic CMMRD testing should be able to offer transcript analysis of all four MMR genes and should be able to apply assays that circumvent potential diagnostic pitfalls	Strong

	that result from the high homology of <i>PMS2</i> and its pseudogene <i>PMS2CL</i> (either by partnership with a different laboratory or in their own laboratory).	
<b>Rec. 10</b>	The laboratory performing genetic CMMRD testing of an index patient with a (pre-)malignancy should probably have one or more validated ancillary assay(s) available (either by partnership with a different laboratory or in their own laboratory) that can definitively confirm or refute the diagnosis of CMMRD if genetic testing renders an inconclusive result (the currently available ancillary assays testing for constitutional MMR deficiency are listed in Table 4).	Strong
<b>Rec. 11</b>	The laboratory performing genetic CMMRD testing of an index patient without a (pre-)malignancy should have one or more validated ancillary assay(s) available (either by partnership with a different laboratory or in their own laboratory) that can definitively confirm or refute the diagnosis of CMMRD if genetic testing renders an inconclusive result (the currently available ancillary assays testing for constitutional MMR deficiency are listed in Table 4).	Strong
	<b>Diagnostic criteria</b>	
<b>Rec. 12</b>	The diagnosis of CMMRD should be considered confirmed in an individual fulfilling one or more of the suggested criteria for CMMRD testing (Rec.1, Rec.2, Rec.3, Rec.4, Rec.6) if, according to the Table "Criteria for the confirmation of CMMRD" (Table 5):  (i) in one of the four MMR genes, two variants classified according to internationally accepted classification criteria* as (likely) pathogenic (PV or LPV) are identified and are	Moderate

confirmed to be located in trans (note that in some cases additional criteria need to be fulfilled);

OR

(ii) in one of the four MMR genes, one of two variants identified and confirmed to be located in trans is classified as a PV or LPV or variant of unknown significance (VUS) and the other one is classified as a VUS and one or more clinically validated ancillary test results is consistent with a CMMRD diagnosis;

OR

(iii) in one of the four MMR genes, one variant is identified and classified as a PV or LPV or VUS and there is evidence for (a) faulty splicing not explained by the identified variant or (b) reduced expression of the wild-type allele by transcript analysis and one or more clinically validated ancillary test results is consistent with a CMMRD diagnosis;

OR

(iv) no MMR gene variant classified as a PV or LPV or VUS is identified, but one or more clinically validated ancillary test results is consistent with a CMMRD diagnosis and there is evidence by transcript analysis for (a) faulty splicing or (b) reduced expression of the wild-type allele(s) of one of the MMR genes.

\*[ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for MMR genes.](#)

<b>Rec. 13</b>	Cancer patients fulfilling the suggested criteria for CMMRD testing, Rec.1, Rec.2 or Rec.4, in whom the diagnosis CMMRD cannot be confirmed, should probably be tested for a germline (likely) pathogenic variant in the exonuclease domains of <i>POLE</i> and <i>POLD1</i> .	Strong
<b>Rec.14</b>	In a deceased cancer patient fulfilling one or more of the suggested criteria for CMMRD testing (Rec.1, Rec.2, Rec.4) for whom no germline DNA/RNA is available and the diagnosis of CMMRD cannot be confirmed by one or more of the criteria outlined in Rec.12 and Table 5, the diagnosis of CMMRD should be considered confirmed if immunohistochemical staining shows expression loss of one or more MMR proteins in neoplastic and in non-neoplastic cells, including tumour infiltrating leukocytes and/or endothelial cells, of the patient and expression in an appropriate positive control.	Moderate

**Table 2: Revised C<sub>4</sub>CMMRD indication criteria for CMMRD testing in cancer patients<sup>+</sup>  
Indication for CMMRD testing in a cancer patient reaching  $\geq 3$  points.**

<b><u>C<sub>4</sub>CMMRD scoring points assigned to (pre-)malignancies in the patient (at least one point is mandatory):</u></b>	
Carcinoma of the Lynch syndrome (LS) spectrum* and/or a high-grade dysplastic adenoma of the digestive tract at age <25 years	3 points
Multiple colorectal adenomas at age <25 years and no genetic diagnosis/explanation upon testing for polyposis syndromes	3 points
T-cell lymphoblastic lymphoma (T-LBL) at age <18 years	2 points
WHO grade III or IV glioma at age <25 years	2 points
Any other malignancy at age <18 years	1 point
<b><u>C<sub>4</sub>CMMRD scoring points assigned to additional features in the patient (optional):</u></b>	
Clinical sign of Neurofibromatosis type 1 (NF1) <sup>§</sup> and/or $\geq 4$ hyperpigmented and/or hypopigmented skin alterations with $\emptyset > 1$ cm	2 points
2 or 3 hyperpigmented and/or hypopigmented skin alterations with $\emptyset > 1$ cm <b>Do not count if two points are already given for "Clinical sign of NF1 and/or <math>\geq 4</math> hyperpigmented and/or hypopigmented skin alterations with <math>\emptyset &gt; 1</math> cm"</b>	1 point
Multiple pilomatrixomas	2 points
One pilomatrixoma	1 point



Agenesis of the corpus callosum	1 point
Non-therapy-induced cavernoma	1 point
Multiple developmental venous anomalies (DVAs, also known as cerebral venous angiomas) in separate regions of the brain	2 points
Paediatric systemic lupus erythematosus	1 point
Deficiency/reduced levels of IgG2/4 and/or IgA	1 point
<b>C<sub>4</sub>CMMRD scoring points assigned to additional features in the family (optional):</b>	
Consanguineous parents	1 point
Diagnosis of LS in a first-degree or second-degree relative	2 points
Carcinoma from LS spectrum* before the age of 60 in a first-degree, second-degree, and/or third-degree relative	1 point
A sibling with a (pre-)malignancy assigned two or three C <sub>4</sub> CMMRD scoring points	2 points
A sibling with any type of childhood malignancy	1 point

**Abbreviations:** C<sub>4</sub>CMMRD = Care for CMMRD; (L)PV(s) = (likely) pathogenic variant(s); WHO = World Health Organization; NF1 = neurofibromatosis type 1.

\*Original C<sub>4</sub>CMMRD criteria: Wimmer et al. Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'care for CMMRD' (C<sub>4</sub>CMMRD). *J Med Genet* 2014; 51(6):355-65.

\*Colorectal, endometrial, small bowel, urothelial, gastric, ovarian, and biliary tract cancer.

§Clinical sign in the patient used for the diagnosis of NF1 according to: Legius et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. *Genet Med* 2021; 23(8):1506-1513.

#Diameter

**Table 3: Selection strategy for CMMRD counselling and testing in a child suspected to have NF1/Legius syndrome (without cancer) and a negative outcome of NF1/SPRED1 germline mutation analysis**

Prerequisites:
<ul style="list-style-type: none"> <li>▶ Suspicion of NF1 due to the presence of at least one diagnostic NF1 feature*, including at least two hyperpigmented skin patches reminiscent of CALMs.</li> <li>▶ No (likely) pathogenic germline variant in <i>NF1</i> and <i>SPRED1</i> detected using comprehensive and highly sensitive mutation analysis protocols#.</li> <li>▶ Absence of diagnostic NF1 sign(s) in both parents.</li> </ul>
Additional features, at least one (either in the family or in the patient) is required:
<p>In the family:</p> <ul style="list-style-type: none"> <li>▶ Consanguineous parents.</li> <li>▶ Genetic diagnosis of Lynch syndrome in one or both parental families.</li> <li>▶ Sibling with diagnostic NF1 sign(s).</li> <li>▶ A (deceased) sibling<sup>§</sup> with any type of childhood malignancy.</li> <li>▶ One of the following carcinomas of the Lynch syndrome spectrum: Colorectal, endometrial, small bowel, urothelial, gastric, ovarian, and biliary tract cancer, before the age of 60 years in a first-degree or second-degree relative.</li> </ul>
<p>In the patient:</p> <ul style="list-style-type: none"> <li>▶ Atypical CALMs (irregular borders and/or pigmentation).</li> </ul>

- ▶ Multiple hypopigmented skin patches.
- ▶ One or more pilomatrixoma(s) in the patient.
- ▶ Agenesis of the corpus callosum.
- ▶ Non-therapy-induced cavernoma.
- ▶ .Multiple developmental venous anomalies (also known as cerebral venous angiomas) in separate regions of the brain.

**Abbreviations:** CMMRD - constitutional mismatch repair deficiency; NF1 - neurofibromatosis type 1; CALMs - café-au-lait macules.

\*Legius et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. *Genet Med* 2021; 23(8):1506-1513.

#Analysis protocol should include methods that identify and/or characterise unusual splice variants.

§This can be expanded to second-degree and third-degree relatives in populations with a high prevalence of founder mutations.

**Table 4: Ancillary tests for assessing constitutional MMR deficiency**

Validated test <sup>#</sup>	CMMRD confirmed	CMMRD refuted
Germline Microsatellite instability (gMSI) testing acc. to Ingham et al. 2013 <sup>a</sup>	gMSI ratios of at least two (usually all three) microsatellite markers are above the validated laboratory's internal thresholds	Not possible by the test
Constitutional MSI (cMSI) testing acc. to Gallon et al. 2019 and 2022 <sup>b</sup>	cMSI score above the validated laboratory's internal thresholds	cMSI score within the score range of negative controls
High-sensitivity MSI (hsMSI) testing acc. to González-Acosta et al. 2020 <sup>c</sup>	hsMSI score above the validated laboratory's internal thresholds	hsMSI score within the score range of negative controls
Ex vivo MSI (evMSI) + methylation tolerance acc. to Bodo et al. 2015 <sup>d</sup>	evMSI <u>and</u> methylation tolerance above the validated laboratory's internal thresholds	evMSI <u>and</u> methylation tolerance within the range of negative controls
MMRDness testing by low-pass whole-genome sequencing/ LOGIC assay in blood leukocytes acc. to Chung et al. 2022 <sup>e</sup>	MMRDness score above the validated laboratory's internal thresholds	MMRDness score within the score range of negative controls

**Abbreviations:** MMR = mismatch repair; CMMRD - constitutional mismatch repair deficiency; MSI = microsatellite instability; acc. = according; PV = pathogenic variant; (L)PV = (likely) pathogenic variant.

<sup>#</sup>Validation cohort should include (i) at least eight CMMRD patients with different genotypes with respect to PVs and affected gene (for each of the four MMR genes at least one patient should be included), (ii) a large number of negative controls consisting of at least twenty adult individuals aged >40 years without cancer

history and without a MMR gene (L)PV, (iii) at least ten confirmed MMR gene PV heterozygotes and, if available, (iv) *POLE* and *POLD1* PV heterozygotes.

<sup>a</sup>Ingham et al. Simple detection of germline microsatellite instability for diagnosis of constitutional mismatch repair cancer syndrome. *Hum Mutat* 2013; 34:847–52.

<sup>b</sup>Gallon et al. A sensitive and scalable microsatellite instability assay to diagnose constitutional mismatch repair deficiency by sequencing of peripheral blood leukocytes. *Hum Mutat* 2019; 40(5):649-655.

<sup>b</sup>Gallon et al. Constitutional microsatellite instability, genotype, and phenotype correlations in Constitutional Mismatch Repair Deficiency. *Gastroenterology* 2022; S0016-5085(22)01444-5. Online ahead of print.

<sup>c</sup>González-Acosta et al. High-sensitivity microsatellite instability assessment for the detection of mismatch repair defects in normal tissue of biallelic germline mismatch repair mutation carriers. *J Med Genet* 2020; 57(4):269-273.

<sup>c</sup>Marín et al. A Validated Highly Sensitive Microsatellite Instability Assay Accurately Identifies Individuals Harboring Biallelic Germline PMS2 Pathogenic Variants in Constitutional Mismatch Repair Deficiency. *Clin Chem* 2024; 70(5):737–746.

<sup>d</sup>Bodo et al. Diagnosis of Constitutional Mismatch Repair-Deficiency Syndrome Based on Microsatellite instability and Lymphocyte Tolerance to Methylating Agents. *Gastroenterology* 2015; 149:1017–29.

<sup>e</sup>Chung et al. Genomic Microsatellite Signatures Identify Germline Mismatch Repair Deficiency and Risk of Cancer Onset. *J Clin Oncol* 2023; 41(4):766-777.

**Table 5: Criteria for the confirmation of CMMRD**

Genotype	MMR gene genetic testing reason					
Germline MMR gene variants identified (if two variants are identified, they must be confirmed to be in trans)	C4CMMRD criteria for cancer patient fulfilled (Rec.1)	Cancer <18 years with paediatric high TMB (Rec.2)	Cancer with MMR protein expression loss in neoplastic and non-neoplastic cells including tumour infiltrating lymphocytes and/or endothelial cells (Rec.3)	Cancer <18 years with heterozygous germline MMR gene (L)PV (Rec.4)	C4CMMRD criteria for children without cancer suspected to have NF1 /Legius syndrome and a negative <i>NF1/SPRED1</i> mutation analysis (Rec.6)	Incidental finding in WES or WGS performed for other reasons in a patient without cancer
PV/PV	√	√	√	√	√	√
PV/LPV	√	√(PPAP-)	√	√	√	√(AT+)
LPV/LPV	√	√(PPAP-)	√(AT+)	√(AT+)	√(AT+)	√(AT+)
(L)PV/VUS	√(AT+)	√(AT+)	√(AT+)	√(AT+)	√(AT+)	√(AT+)
VUS/VUS	√(AT+)	√(AT+)	√(AT+)	NA	√(AT+)	NA

(L)PV/X	√(AT+;mRNA+)	√(AT+;mRNA+)	√(AT+;mRNA+)	√(AT+;mRNA+)	√(AT+;mRNA+)	NA
VUS/X	√(AT+;mRNA+)	√(AT+;mRNA+)	√(AT+;mRNA+)	NA	√(AT+;mRNA+)	NA
X/X	√(AT+;mRNA+)	√(AT+;mRNA+)	√(AT+;mRNA+)	NA	√(AT+;mRNA+)	NA

**Abbreviations:** C<sub>4</sub>CMMRD = Care for CMMRD; MMR = mismatch repair; TMB = tumour mutation burden; PV = pathogenic variant; LPV = likely pathogenic variant; (L)PV = likely pathogenic or pathogenic variant; VUS = variant of unknown significance; WES = whole exome sequencing; WGS = whole genome sequencing; alleles are separated by / and X indicates one allele without an identifiable (L)PV or VUS.

NA = not applicable

√ = CMMRD confirmed without further ancillary test or transcript analysis

√(PPAP-) = CMMRD confirmed without further ancillary test if POLE /POLD<sub>1</sub> germline mutation excluded (i.e. polymerase proofreading associated polyposis negative: PPAP-)

√(AT+) = CMMRD confirmed if validated ancillary test positive for CMMRD (AT+)

√(AT+;mRNA+) = CMMRD confirmed if validated ancillary test positive for CMMRD(AT+) and evidence by transcript analysis for (a) faulty splicing (not explained by the identified variant) or (b) reduced expression of the wildtype allele(s) (mRNA+)

## 9.2 GENETIC COUNSELLING - SUMMARY OF EVIDENCE AND GUIDELINE RECOMMENDATIONS

Genetic counselling should be recommended to the family of a CMMRD patient and it should include information about the potential 25% recurrence risk in siblings and the risk of Lynch syndrome (LS)-associated cancer development in both parents and other relatives at risk of being affected by LS (Rec. 1). Cascade testing must be offered to relatives including parents (Rec. 2-3) as they also need to be informed about LS surveillance programs (Durno et al., 2017a; Suerink et al., 2019b; Wimmer et al., 2014).

Genetic counselling for siblings should be offered by a multidisciplinary team consisting of a paediatric oncologist, a medical geneticist and a psychologist (Wimmer et al., 2014) (Rec. 1). Besides family screening, education about the disease should also be offered to the family (Kebudi et al., 2020). For siblings, the proposal of a genetic test can be done at any age so that early surveillance can be offered if CMMRD is identified (Rec. 4). However, one should not ignore the fact that testing young siblings may reveal LS in a minor despite there being no recommendations to offer surveillance measures at this age. This issue should be discussed with the parents before the genetic test is performed (Rec. 5).

If the diagnosis of CMMRD is not confirmed by the identification of two (likely) pathogenic variants in one MMR gene but is confirmed by ancillary tests in the patient, siblings may also be tested by ancillary tests to confirm or exclude CMMRD (Rec. 6). Ancillary tests may also help to rule out a diagnosis of CMMRD in a child with mild clinical features of CMMRD, such as e.g. four or more CALMs and a known diagnosis of LS in one parent (Rec. 12), to avoid revealing LS status during childhood. There are no published examples of this type of situation, but some were reported and discussed at the C<sub>4</sub>CMMRD Consortium conference on 16 November 2022 in Paris. A diagnosis of CMMRD will affect family planning and future reproductive decisions of the parents as well as of the CMMRD patient if they are of, or when they reach, reproductive age. Pre-implantation genetic testing or prenatal testing is possible when the biallelic MMR PVs are identified (Durno et al., 2017a; Durno et al., 2015) and should be discussed with parents of a CMMRD patient (Rec. 7) and with couples of reproductive age if both of them carry a pathogenic variant in the same MMR gene (Rec. 8). In France, there has been one birth following preimplantation genetic diagnosis for a couple in this situation (Corsini C., Montpellier, personal data reported during C<sub>4</sub>CMMRD meeting in Paris, Nov 2022).

There is discussion of whether LS carriers of reproductive age should be informed about the risk of CMMRD syndrome for their offspring and of the possibility of testing their partner before pregnancy. This is particularly relevant for the *PMS2* gene since, based on data from first degree relatives in the Colon Cancer Family Registry, the frequency of *PMS2* PV carriers in the general population is 1 in 714 and they may not present as LS due to the low penetrance of *PMS2* PV in the heterozygous state (Win et al., 2017). Therefore, the *a priori* risk for a *PMS2*-associated LS carrier to give birth to a child affected with CMMRD is estimated to be 1/2856 ( $1/4 \times 1/714$ ) and for a *PMS2*-associated CMMRD patient it is estimated to be 1/1428 ( $1/2 \times 1/714$ ). These risks are higher in cases of consanguinity of the couple or in populations with founder effects (Goldberg et al., 2015).

Currently, there are no published recommendations regarding whether to test the partner of a Lynch or CMMRD syndrome patient outside the context of consanguinity or founder effect. This question was discussed at the last C<sub>4</sub>CMMRD Consortium meeting in November 2022 in Paris without reaching a consensus. Indeed, such a recommendation depends on the

possibilities of access to genetic testing in each country (costs, prescription habits, and access to genetic counselling). Furthermore, it should be considered that a complete analysis of an MMR gene in the partner could reveal a VUS making genetic counselling complicated and that comprehensive *PMS2* analysis, as explained above, remains complex and limited to certain laboratories. The recommendations given here (Rec. 9-11) rely entirely on the Delphi process, which reached a consensus that genetic testing of MMR genes should not be offered to partners of an LS carrier in the absence of consanguinity, the partner belonging to a population with a known founder variant, or the partner having a family history suggestive of LS. However, testing should be offered to the partner of an LS carrier in the presence of any of these three criteria, as well as to partners of CMMRD patients.

Recommendations		Strength
<b>Rec. 1</b>	Genetic counselling should be offered to parents and siblings of a confirmed CMMRD patient, preferentially by a multidisciplinary team with knowledge of CMMRD, consisting of a medical geneticist, a paediatric oncologist and a psychologist.	Strong
<b>Rec. 2</b>	To confirm their carrier status, parents of a CMMRD patient should be offered genetic testing for the (likely) pathogenic MMR gene variants found in their child.	Strong
<b>Rec. 3</b>	Cascade genetic testing for (likely) pathogenic variants should be offered to all adult relatives of a CMMRD patient, in both parental branches.	Strong
<b>Rec. 4</b>	Siblings of a genetically confirmed CMMRD patient should be offered genetic CMMRD testing regardless of age and phenotype.	Strong
<b>Rec. 5</b>	When performing CMMRD predictive testing in a minor or prenatal testing, pros and cons of revealing results of genetic testing regarding Lynch syndrome should be discussed on a case-	Moderate

	by-case basis with the parents and the patient depending on their age.	
<b>Rec. 6</b>	If the diagnosis of CMMRD is not confirmed by the identification of two (likely) pathogenic variants in one MMR gene but by ancillary tests in the patient, siblings should probably be offered ancillary tests to exclude a CMMRD diagnosis for them.	Moderate
<b>Rec. 7</b>	Prenatal or preimplantation genetic testing should be discussed with parents of reproductive age of a CMMRD patient.	Strong
<b>Rec. 8</b>	Prenatal or preimplantation genetic testing should be discussed with couples of reproductive age if both carry a pathogenic variant in the same MMR gene.	Strong
<b>Rec. 9</b>	Testing the partner of a CMMRD patient for the MMR gene involved should probably be discussed during genetic counselling, considering possible consanguinity, common founder effect, and family history suggestive of Lynch syndrome.	Strong
<b>Rec. 10</b>	The partner of a Lynch syndrome carrier should be offered genetic testing of MMR genes if consanguinity is reported by the couple or the partner is coming from a population with a known founder variant or the family history of the partner is suggestive of Lynch syndrome and genetic testing has not been performed yet.	Strong
<b>Rec. 11</b>	The partner of a Lynch syndrome carrier should not be actively offered genetic testing of MMR genes in the absence of consanguinity, a known founder mutation or a family history suggestive of Lynch syndrome.	Moderate
<b>Rec. 12</b>	The child of a Lynch syndrome carrier should probably be offered CMMRD testing, if the child has clinical features that add up to $\geq 2$	Strong

	C4CMMRD scoring points according to the revised criteria (Table 2: scoring points assigned to additional features in the patient).	
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### 9.3 SURVEILLANCE - SUMMARY OF EVIDENCE AND GUIDELINE RECOMMENDATIONS

CMMRD patients have a high risk of malignancies from their first year and throughout their life. The tumour spectrum includes primarily haematological malignancies, brain tumours and intestinal tract tumours. Three main publications propose guidelines for surveillance of CMMRD patients based on expert opinions and available data of the frequencies and the age at diagnosis of different tumour entities. These are: the European C4CMMRD Consortium (Vasen et al., 2014), the US Multi-Society Task Force on Colorectal Cancer (Durno et al., 2017a) and the Pediatric Cancer Working Group of the American Association for Cancer Research (AACR) (Tabori et al., 2017). Of note, these recommendations were published several years ago and were based on limited evidence. An overview of these surveillance protocols can be found in section 10 Table 6.

Although a randomized controlled trial would be needed to determine the extent to which surveillance recommendations improve the prognosis of patients with CMMRD, ethical considerations and the limited number of patients make such a study unfeasible. As a result, current evidence for the efficacy of surveillance in CMMRD patients is drawn from studies of other cancer predisposition syndromes such as Lynch syndrome and Li-Fraumeni syndrome, as well as from observational prospective studies in individuals with CMMRD conducted by the European C4CMMRD consortium and the IRRDC. These studies have demonstrated a survival benefit for those individuals with CMMRD who undergo surveillance compared to those who do not (Durno et al., 2021; Ghorbanoghli et al., 2023).

It is widely accepted that CMMRD patients and their parents should be educated about tumour risks associated with CMMRD and about symptoms related to the main tumours especially dyspnoea and superior vena cava syndrome for mediastinal lymphoma, symptoms associated with pancytopenia (e.g. bruising, recurrent infections, pallor, fatigue, etc.) for leukaemia, neurological symptoms for brain tumours, and bleeding for colorectal tumours



(Rec. 1-2). The three major international groups also consistently recommend a clinical examination for children and adults with CMMRD every 6 months (Rec. 5).

Pros and cons of more specific surveillance modalities should be discussed with the CMMRD patient and/or their parents so that they, together with the clinician, can make an informed joint decision to participate in a surveillance program (Rec. 3). No direct evidence of the health risks associated with surveillance is reported in the literature. Nonetheless, some potential risks should be discussed: in particular, the specific adverse events of each procedure and complications of general anaesthesia necessary for some recommended examinations (MRI in young children, digestive tract endoscopies).

A greater awareness of being at high risk for developing cancers may increase psychological distress before and after surveillance examinations. In addition, examinations may reveal small lesions of unknown significance in asymptomatic patients for which the only management option is monitoring at a short follow-up interval, which may increase anxiety.

CMMRD patients and their families should also be encouraged to participate in research projects evaluating the surveillance programs and to register in a database to improve knowledge of CMMRD (Rec. 4).

### **Overall effectiveness of the previously proposed surveillance modalities**

The C<sub>4</sub>CMMRD Consortium reported prospective follow-up results of 22 patients undergoing their proposed surveillance protocols (Ghorbanoghli et al., 2023). During a mean follow-up of 4 years, the program detected nine asymptomatic malignant tumours including three brain tumours, three upper gastrointestinal tract cancers and three colorectal cancers. Most tumours were successfully treated. In addition, many adenomas of the duodenum and colorectum were detected and subsequently removed. At the end of the follow-up period, 16 out of 22 patients (73%) who participated in the surveillance program were still alive.

In 2015, the IRRDC described preliminary evidence that supported the benefit of surveillance protocols to CMMRD patients (Durno et al., 2015), and subsequently published an observational study that provides the best evidence for the efficacy of the different

modalities to date (Durno et al., 2021). In this study, a total of 191 malignant tumours in 110 patients were identified. For patients undergoing surveillance according to the protocol proposed by the Paediatric Cancer Working Group of the AACR (Tabori et al., 2017), 100% of gastrointestinal and other solid tumours, as well as 75% of the brain cancers were detected in asymptomatic patients. In contrast, only 16% of haematological malignancies were detected by screening. Of note, among the 191 tumours reported in this study, only 12 (6%) were outside the classical spectrum of CMMRD.

Taken together, both studies show that surveillance of the digestive tract and the brain led to early detection of tumours supporting the effectiveness of the suggested surveillance measures (Durno et al., 2021; Ghorbanoghli et al., 2023). Haematological tumours were mainly discovered incidentally and between follow-up examinations. Hence, monitoring for haematological malignancies has not proven to be effective.

### **Overall survival benefit of the surveillance modalities**

The IRRDC study included a prospective cohort of 89 patients under full, partial, or no surveillance. The prospective observational data showed that a patient's 5-year overall survival (OS) was significantly higher when an asymptomatic cancer was detected compared to a symptomatic cancer (90% vs 50%,  $p=0.001$ ). Subgroup analysis found the 5-year OS of central nervous system tumour patients was significantly higher when the tumour was detected asymptotically compared to symptomatic tumours (72% vs 33%,  $p=0.04$ ). For gastrointestinal cancer patients, the 5-year OS for tumours detected asymptotically was superior to symptomatic tumours, although this difference did not reach statistical significance (100% vs 81%,  $p=0.18$ ) (Durno et al., 2021).

Survival was significantly different depending on the extent of the surveillance with 4-year overall survival being 79% for those under full surveillance compared to 54% for those under partial surveillance ( $p<0,0001$ ) and 15% for those who did not follow surveillance ( $p<0,001$ ). In multivariable analysis, including age, sex, gene affected, and resources available, surveillance was the single variable associated with improved OS in patients with CMMRD

( $p=0.0001$ ). However, no statistically significant difference was observed in mortality between the full surveillance and partial surveillance cohorts (Durno et al., 2021).

A further outcome assessed in this report is the likelihood of transformation of low-grade CMMRD tumours to high-grade cancers. Of the 64 low-grade tumours detected, the cumulative likelihood of transformation to high-grade cancer was 81% for gastrointestinal cancers within 8 years and 100% for gliomas within 6 years (Durno et al., 2021).

### **Brain tumours**

Brain tumours are the most frequent tumour in CMMRD patients accounting for 35% (Wimmer et al., 2017b) to 44% (Aronson et al., 2022) of all malignancies and occurring in over half of patients (Guerrini-Rousseau et al., 2019; Rigaud et al., 2024; Wimmer et al., 2017b). In addition, HGGs are the main cause of death in these patients (Guerrini-Rousseau et al., 2019; Lavoine et al., 2015).

The three major international groups providing recommendations for CMMRD surveillance agree on the importance of brain MRI for early detection. They recommend that brain MRI should be performed at the time of CMMRD diagnosis or no later than age two years (Durno et al., 2017a; Tabori et al., 2017; Vasen et al., 2014), with consensus on an interval of every six months in children (C4CMMRD proposes every 6-12 months) (Rec. 6-7).

Both the IRRDC study and the C4CMMRD study evaluating the previous guidelines support a six-month interval for brain MRI. Only six out of 24 brain tumours found in patients undergoing brain tumour surveillance were detected symptomatically, and these six tumours were found in patients who had longer brain MRI intervals (1 year, 1.5 years, and 8 months) or interruptions in their surveillance (Durno et al., 2021; Ghorbanoghli et al., 2023). The majority of the published CMMRD patients developed brain tumours in childhood and adolescence (mean age at diagnosis of 9-10 years, range 2-40 years) (Durno et al., 2021; Wimmer et al., 2014). As most CMMRD-patients do not reach adulthood, we have no studies assessing the brain tumour risk in an adult population. Nonetheless, given their already intensive surveillance program, the recommendation to perform annual screening in adults from the age of 20 years by brain imaging achieved consensus (Rec. 8).

There is no consensus on the MRI sequences to be performed for the screening of brain tumours associated with CMMRD. Except for patients who already have a brain tumour, contrast enhancement injection is recommended only for the first MRI. To avoid intracerebral accumulation of contrast products, the following MRIs can probably be offered without injection. Paediatric neuro-radiologists of the French paediatric oncology society (SFCE) recommend anatomical sequences T2-FLAIR (if possible in 3D) combined with a MRI diffusion sequence. In case of a doubtful cerebral lesion, an injection is recommended. (Rec. 9-11).

### **Leukaemia and lymphoma**

Lymphoid and other haematological malignancies are among the most common malignancies observed in children with CMMRD, occurring in about one third of CMMRD patients. In total, they account for about 20% of CMMRD-associated tumours (Durno et al., 2021; Wimmer et al., 2014). Lymphomas are the most frequent (around two thirds of haematological malignancies) and analysis of patients registered in the C<sub>4</sub>CMMRD and IRRDC databases shows that most haematological malignancies are of T-cell origin and 82% of those present as T-lymphoblastic lymphoma (T-LBL) (Rigaud et al., 2024; Ripperger & Schlegelberger, 2016). Leukaemias are less frequent in CMMRD accounting for only a third of haematological malignancies and less than 7% of all malignancies (Durno et al., 2021; Wimmer et al., 2014) including mostly acute lymphoblastic leukaemia and acute myeloid leukaemia. The median age at diagnosis is 8 years for leukaemia according to compiled data of CMMRD patients (Durno et al., 2017a). In the C<sub>4</sub>CMMRD database, around 100 patients were included before 2022 and nine experienced a leukaemia: four with ALL, four with AML and one with chronic myeloid leukaemia. The median age at diagnosis was four years (1.7-12 years) (unpublished data).

Guidelines for lymphoma surveillance of the three previous publications are very heterogeneous (see also Table 5 in section 10). The Pediatric Cancer Working Group of the AACR recommends an abdominal ultrasound every 6 months starting at the age of one (Tabori et al., 2017). In addition, it has been proposed that annual whole-body MRI (WBMRI) could be alternated with abdominal ultrasound (Tabori et al., 2017). By contrast, the

European C<sub>4</sub>CMMRD Consortium recommends clinical examination every 6 months starting at the age of one year, with abdominal ultrasound as an optional intervention (Vasen et al., 2014). For leukaemia, all groups had agreed on the recommendation of a complete blood count every 6 months and starting at the age of one year (Aronson et al., 2022).

The prospective study of the IRRDC did not find an impact of these screening guidelines on early diagnosis or outcome of haematological malignancies in CMMRD patients (Durno et al., 2021). Considering the rapid tumour growth of paediatric NHL, screening requires short intervals between evaluations, which should be performed at least every 3 months. In addition, abdominal ultrasound is not able to detect T cell lymphoma, which is the most frequent NHL subtype in CMMRD. Regular chest X-rays are not recommended for screening because of the potential genotoxic effects of repeated exposure to X-rays.

While blood count may be useful in cases of bone marrow infiltration, it has limited value in ALL or NHL surveillance (Porter et al., 2017). Although regular blood sampling for detection of circulating T-cell rearrangement may be a potential option for early diagnosis of T lymphoblastic NHL, its effectiveness in CMMRD is unknown and needs further evaluation in research studies. This is in agreement with a review, where the effectiveness of haematological screening was questioned because NHL and ALL are rapidly growing tumours and monitoring may not improve outcomes for these patients (Westdorp et al., 2017).

Based on these observations and considerations, we recommend only clinical monitoring every 6 months (Rec. 5) and do not recommend abdominal ultrasound or systematic blood count (Rec. 12-13).

## **Lynch syndrome-related tumours**

### **Colorectal cancer**

Among Lynch syndrome-related tumours, colorectal cancers are the most frequent in CMMRD with a median age of onset of 16 years (range 8-48 years) (Aronson et al., 2016;

Durno et al., 2017b; Wimmer et al., 2014). The CMMRD intestinal tract phenotype also includes adenomatous polyps ranging from development of a single adenoma to polyposis. All expert groups agree on annual colonoscopy as the most effective intervention as it facilitates both early cancer detection and polyp removal before progression into cancer. The prospective observational studies of both the IRRDC group and C4CMMRD Consortium confirmed the effectiveness of colonoscopy (Durno et al., 2021; Ghorbanoghli et al., 2023). However, there are some differences in previous guidelines regarding the starting age for colonoscopic surveillance. Based on the earliest age of onset in 53 CMMRD patients with one or more colorectal cancers, the European C4CMMRD consortium recommended starting surveillance colonoscopy at the age of 8 years (Wimmer et al., 2014). Levi et al. also suggest starting at 8 years, because no significant gastrointestinal findings were made prior to this age in their 11-case series (Levi et al., 2015). In contrast, (Herkert et al., 2011) suggest yearly colonoscopy starting at age 6 years as first adenomas were identified at age 7 years in one of their patients (Herkert et al., 2011). Durno et al. recommend starting at age 6 years (Durno et al., 2017a) and Tabori et al. recommend starting at age 4 to 6 years due to reports of colonic polyps at 6 years of age (Tabori et al., 2017). Prospective evaluation of the surveillance benefits revealed no cases of colorectal cancer below the age of 8 years (Durno et al., 2021; Ghorbanoghli et al., 2023). The current data of the C4CMMRD database reports the earliest colorectal cancer at the age of 7.5 years and earliest adenoma at the age of 6 years. Therefore, colonoscopy should start at an age of 6 years to detect adenomas that may progress to cancer (Rec. 14). The progression of adenomas to malignancy in CMMRD is one of the most rapid of any inherited colorectal cancer syndrome (Aronson et al., 2016; Shlien et al., 2015), and surveillance intervals should, therefore, not exceed 1 year (Rec. 20). Increasing the colonoscopy frequency from a 1 year to an approximately 6-months interval once polyps are detected is recommended (Rec. 18), with resection of all lesions, irrespective of size. In cases of polyposis with high-grade dysplasia or cancer or too many polyps to remove by endoscopy, surgical intervention is recommended with (sub)total colectomy with ileorectal anastomosis, followed by close endoscopic monitoring of the rectum every 6 to 12 months (Section 9.5 – Rec. 17). Ileal pouch-anal anastomosis may be necessary depending on rectal polyp burden. If the monitoring schedule or surgical modalities are different, they must be discussed by a specialist board.

The surveillance guidelines of the European C<sub>4</sub>CMMRD Consortium also suggested that a paediatric gastroenterologist should perform the procedure together with an adult gastroenterologist with experience of endoscopic mucosal resection of large non-polypoid lesions (Vasen et al., 2014). More generally, the European Society of Gastrointestinal Endoscopy (ESGE) recommends that individuals with Lynch syndrome should be followed in dedicated units that practice monitoring of compliance and endoscopic performance measures with high quality surveillance colonoscopy (van Leerdam et al., 2019). This is equally advisable for CMMRD patients (Rec. 19).

No data is available concerning the value of using a virtual or indigo carmine coloration during colonoscopy in CMMRD patients. Although some publications have suggested that such coloration may improve detection of flat adenomas in Lynch syndrome (Har-Noy et al., 2019; Perrod et al., 2021), this is still a matter of debate. In familial adenomatous polyposis, endoscopy with coloration may also help to determine the exact polyp burden in both the colorectum and duodenum to inform surgical decisions (Huneburg et al., 2020; Matsumoto et al., 2009). Therefore, it is recommended that both upper endoscopy and colonoscopy should probably be done with (virtual) coloration in the context of CMMRD (Rec. 17).

### **Upper gastro-intestinal lesions**

Early onset adenomas and cancers of the small bowel and stomach are described in patients with CMMRD. Levi and colleagues (Levi et al., 2015) report tumours in the upper gastrointestinal tract in two of 11 CMMRD patients. In the review by Durno et al. (Durno et al., 2017a), one half of CMMRD patients had small bowel adenomas, diagnosed at a median age of 12 years (range 10-20 years). In a cohort of 24 CMMRD patients with available data of the digestive tract, four had small bowel cancer at a median age of 18 years (range 11-42 years) (Aronson et al., 2016; Durno et al., 2017a). In the C<sub>4</sub>CMMRD database, three of 36 patients with data of the digestive tract available had a small bowel cancer at 21, 26 and 44 years of age (unpublished data).

During prospective follow up of 20 CMMRD patients, five patients had duodenal adenomas at a median age of 18.6 years (range: 10.1-28.1 years), two had gastric cancer (the youngest

at 10 years of age was diagnosed during surveillance 16 months after their previous upper gastrointestinal tract endoscopy), one oesophageal cancer and one small bowel (jejunal) cancer (Ghorbanoghli et al., 2023). All upper gastro-intestinal lesions were found during surveillance. Only one patient, not under surveillance, developed a symptomatic small bowel cancer at age 26 (Ghorbanoghli et al., 2023).

Among the 110 patients included in the full study cohort of the IRRDC assessment of surveillance guidelines, nine patients developed small bowel cancers between 9 and 33 years of age, three patients developed stomach cancer at 9, 25, and 33 years, and two patients developed biliary tract cancers at 13 and 22 years (Durno et al., 2021).

Annual video capsule endoscopy (VCE) and gastroscopy are the interventions recommended by the two expert groups, although some differences in the guidelines are found. The European C4CMMRD Consortium recommends starting VCE and gastroscopy at the age of 10 years, while the IRRDC recommends starting at the age of 8 years (see table 2 in (Aronson et al., 2022)). Herkert et al. also suggested yearly endoscopy and VCE from age 8 years (Herkert et al., 2011). An additional recommendation from the IRRDC is to increase the gastroscopy to every 6 months when polyps are identified. Very young children are generally able to swallow the capsule unaided, but those who are undergoing endoscopic surveillance under general anaesthesia can have the capsule placed endoscopically at the same time (Herkert et al., 2011; Vasen et al., 2014). Given the diagnoses of small bowel and stomach cancers in CMMRD patients as young as 9 years, it is recommended VCE and gastroscopic surveillance are started at the same time as colonoscopic surveillance, or at age 10 years at the latest, and performed annually with increased frequency to 6-month intervals after detection of polyps (Rec. 15, 18, 21)

It is important to note some limitations of VCE for small-bowel surveillance. In a report from the IRRDC the authors mention the potential for incomplete studies, a high rate of false positive examinations and the possibility of a false negative examination. They also recommend considering additional endoscopic modalities to complement VCE, such as push enteroscopy with careful inspection of the ampullary region because small bowel neoplasms are often proximally located and may be missed on VCE (Shimamura et al., 2018) (Rec. 16).



MRI of the small bowel is an alternative to VCE, but there are no published data on its use in patients with CMMRD. It has therefore not been included in the recommendations, but could be considered as part of a case-by-case discussion.

### **Gynaecological and urinary tract tumours**

The incidence of gynaecological and urinary tract tumours in CMMRD is unknown, especially as the majority of these tumours in CMMRD have been reported at an age that most CMMRD patients do not reach. Very few such tumours have been described so far with an early age of onset. Specifically, there are fewer than ten patients with renal or urinary tract cancers, which were diagnosed from age 11 to 22 years. There are also fewer than ten endometrial cancers, which were diagnosed from age 19 to 44 years (Durno et al., 2017a). Otherwise, there have been singular cases of an ovarian cancer at age 17 years (Vaughn et al., 2010), endometrioid adenocarcinomas affecting both ovaries at age 26 years (Ramchander et al., 2017), an ovarian neuroectodermal tumour at age 21 years, and endometrial adenocarcinoma of the uterus and ovary at age 23 years (Trimbath et al., 2001).

Both the European C4CMMRD Consortium and the IRRDC follow recommendations proposed for Lynch syndrome carriers for urinary tract surveillance albeit starting at an earlier age, which include annual urine cytology and urine dipstick from the age of 20 years. The US Multi-Society Task Force recommends annual urine analysis starting at the age of 10 years and to consider MRI. It should be noted that numbers to test the effectiveness of these surveillance measures in CMMRD are much too small. Furthermore, for Lynch syndrome, there is no consensus on screening protocols for urinary tract tumours and none of these measures have been proven to be effective.

For gynaecological surveillance, expert groups recommend annual gynaecological examination, transvaginal ultrasound and endometrial biopsy starting at the age of 20 years.

As very few female CMMRD patients reach an age at which they have completed childbearing, recommendations for risk-reducing hysterectomy and salpingo-oophorectomy do not exist. However, as for Lynch syndrome, this could be discussed after completion of childbearing (Rec. 23).

The two prospective evaluations of surveillance efficacy (Durno et al., 2021; Ghorbanoghli et al., 2023) did not specifically evaluate these recommendations for gynaecological and urinary tract tumours. Five patients had urinary tract tumours: two nephroblastomas, which are not part of the LS spectrum, at three and four years, two transitional renal cell carcinomas at 11 and 43 years, and one papillary transitional cell carcinoma at 15 years of age. Another one developed a bladder cancer at 48 years, and one a uterus cancer at 30 years. Only eight patients were older than 18 years at the beginning of the surveillance periods across both studies.

Expert advice during the Delphi process was to not recommend annual urine cytology and urine dipstick to CMMRD patients because their benefit has not been demonstrated (Rec. 24). The same decision was made for endometrial biopsy. However, experts stated that abdomino-pelvic ultrasound for gynaecological and urinary tract cancer screening, as well as clinical examination and transvaginal ultrasound for gynaecological surveillance should probably be offered annually, starting at 20 years of age (Rec. 22, 25).

As breast cancer is rarely reported in CMMRD patients (two cases at 22 and 30 years of age are reported in (Durno et al., 2021)), there is currently no indication for an increased breast cancer risk and need for specific breast cancer screening in CMMRD. Hence, we propose to follow general population guidelines for monitoring (Rec. 26).

### **Whole-body MRI**

The latest published recommendations by the Pediatric Cancer Working Group of the AACR introduced a WBMRI once a year starting at the age of 6 years as a potentially useful screening tool with the advantage of not requiring anaesthesia (Tabori et al., 2017). Considering the tumour spectrum in CMMRD, WBMRI may not have the same efficacy as in Li-Fraumeni syndrome, where sarcomas are more frequent (Ballinger et al., 2017; Frebourg et al., 2020; Tewattanarat et al., 2022). Data to assess its efficacy as a surveillance tool in CMMRD patients are lacking. In the publication reporting the IRRDC experience, only one tumour (type not specified) of 193 was detected by WBMRI (Durno et al., 2021). We considered the data currently insufficient to draw conclusions on the effectiveness of

WBMRI. We thus propose to include surveillance by WBMRI as optional in the recommendations except for the recommendation to offer WBMRI at least once at diagnosis or when the young patient no longer needs general anaesthesia for MRIs to detect malformations and low-grade tumours requiring resection or adapted surveillance (Rec. 27-29). Only an analysis of a large series of patients with a surveillance program including WBMRI will allow to assess the value of this exam for CMMRD, thus we encourage collecting data on WBMRI so that its benefit in CMMRD can be assessed. As brain tumours are the major oncological risk in CMMRD patients, WBMRI should not replace specific brain imaging.

**Cost, cost-effectiveness, invasiveness, acceptability of surveillance interventions**

No information is available in literature regarding cost, cost-effectiveness, invasiveness or acceptance of the different surveillance interventions.

Recommendations		Strength
<b>Rec. 1</b>	CMMRD patients and/or their parents should be educated about tumour risks associated with CMMRD.	Strong
<b>Rec. 2</b>	CMMRD patients and/or their parents should be educated about symptoms related to the main tumours, especially dyspnoea and superior vena cava syndrome for mediastinal lymphomas, symptoms associated with pancytopenia for leukaemia, neurological symptoms for brain tumours, and bleeding for colorectal tumours.	Strong
<b>Rec. 3</b>	Pros and cons should be discussed among the CMMRD patient and/or their parents and clinician to make a joint decision to participate in a surveillance program.	Strong

<b>Rec.4</b>	CMMRD patients and/or their parents should probably be encouraged to communicate their screening results in research projects or databases to improve knowledge on CMMRD.	Strong
<b>Rec. 5</b>	In children and adults with CMMRD, clinical examination should be performed every 6 months.	Strong
<b>Rec. 6</b>	Brain MRI should probably start at the initial CMMRD diagnosis or at least at the age of 2 years.	Strong
<b>Rec. 7</b>	In CMMRD patients up to age 20 years, brain MRI should be performed every 6 months.	Strong
<b>Rec. 8</b>	In CMMRD patients older than 20 years, a brain MRI should be performed at least annually.	Moderate
<b>Rec. 9</b>	The first brain MRI should probably be performed with contrast enhancement for all CMMRD patients.	Moderate
<b>Rec. 10</b>	In patients with CMMRD without a previous brain tumour, MRI should probably include anatomical sequence T2 FLAIR (if possible in 3D) combined with MRI diffusion sequence.	Moderate
<b>Rec.11</b>	In patients with CMMRD with a previous brain tumour, MRI should include anatomical sequences T2-FLAIR, diffusion sequence, and T1+ contrast enhancement if possible in 3D.	Moderate
<b>Rec. 12</b>	Abdominal ultrasound should probably not be performed to screen for abdominal lymphomas in CMMRD patients.	Weak
<b>Rec. 13</b>	Blood counts should probably not be performed to screen for haematological (pre-)malignancies in CMMRD patients.	Weak
<b>Rec. 14</b>	Colonoscopy should be performed at least annually in CMMRD patients and should probably start from the age of 6 years in children with CMMRD.	Strong

<b>Rec. 15</b>	Upper gastrointestinal endoscopy should be performed annually in CMMRD patients and should probably start at the same age as colonoscopy or at least at the age of 10 years.	Strong
<b>Rec. 16</b>	Upper endoscopy should probably use push enteroscopy and careful inspection of the ampullary region in CMMRD patients.	Moderate
<b>Rec. 17</b>	Upper endoscopy and colonoscopy should probably be done with coloration in the context of CMMRD.	Strong
<b>Rec. 18</b>	The frequency of upper or lower endoscopy should probably increase up to 6 months-interval once polyps are detected in the context of CMMRD.	Strong
<b>Rec. 19</b>	Digestive tract surveillance for CMMRD patients, including children, should probably be done in a centre with gastroenterologists experienced in Lynch syndrome screening.	Moderate
<b>Rec. 20</b>	The interval between two digestive tract examinations should not exceed 12 months for CMMRD patients.	Strong
<b>Rec. 21</b>	Video capsule endoscopy should be performed annually in CMMRD patients and should probably be performed from the age of 10 years.	Strong
<b>Rec. 22</b>	Gynaecologic surveillance should probably be performed annually from age 20 years in CMMRD patients and should include clinical examination and transvaginal ultrasound.	Strong
<b>Rec. 23</b>	Prophylactic hysterectomy should probably be discussed once family planning of the CMMRD patient is completed.	Moderate
<b>Rec. 24</b>	Annual urine cytology and urine dipstick should probably not be offered to CMMRD patients.	Moderate

<b>Rec. 25</b>	Abdominopelvic ultrasound for gynaecological and urinary tract cancer screening should probably be offered annually to CMMRD patients, starting at 20 years of age.	Strong
<b>Rec. 26</b>	Breast cancer screening should probably follow general population guidelines for CMMRD patients.	Moderate
<b>Rec. 27</b>	Whole body MRI should probably be offered to CMMRD patients at least once, at diagnosis or when anaesthesia is no longer required, for a general screening of low-grade tumours and malformations to guide targeted screening.	Strong
<b>Rec. 28</b>	Resection or specific surveillance of low-grade lesions should be offered to CMMRD patients.	Strong
<b>Rec. 29</b>	Even though evidence of its efficacy in screening is still weak in CMMRD, whole-body MRI should probably be discussed with CMMRD patients as an option for annual surveillance.	Moderate

#### 9.4 QUALITY OF LIFE - SUMMARY OF EVIDENCE AND GUIDELINE RECOMMENDATIONS

There are no available studies specifically addressing the quality of life of CMMRD patients and their family, which is needed to better understand the impact of the diagnosis and of living with CMMRD.

Most authors who briefly address this topic in case reports, reviews or consensus statements agree that the diagnosis of CMMRD in a child has important implications not only for the child, but also for the entire family (Baig et al., 2019; Biller et al., 2016; Durno et al., 2015; Ozyoruk et al., 2021; Ramchander et al., 2017; Ripperger & Schlegelberger, 2016; Wimmer et al., 2014; Wimmer et al., 2017b).

Healthcare professionals need to understand and address the psychosocial implications of genetic testing for CMMRD to best offer psychological support to the patient and their family

and to avoid refusal of medical care (Urganci et al., 2015). For this, psychological support should be offered to the patient and the family during the entire process of diagnostic evaluation (Ramchander et al., 2017; Suerink et al., 2021a; Wimmer et al., 2014; Wimmer et al., 2017b). The family needs to be aware of the implications of the test result and of the high risk of multiple malignancies in a CMMRD patient (Wimmer et al., 2014). Moreover, because surveillance does not guarantee prevention of cancer, it may cause a great psychological burden in families with a CMMRD child (Ozyoruk et al., 2021). The vast majority of individuals will likely experience specific worries about CMMRD and about how to cope with a cancer diagnosis and cancer risk in their family (Suerink et al., 2021a).

Regarding parents of CMMRD children, a case report by Bruwer et al. (2014) stated that knowing the test results of their children helped them alleviate the uncertainty and anxiety associated with the unknown, and that they felt some sense of empowerment by being informed (Bruwer et al., 2014).

There is no available publication regarding compliance with surveillance programs of CMMRD patients.

Recommendations		Strength
<b>Rec. 1</b>	Psychological support should be offered to the patient and the family during the entire process of evaluation before the diagnosis of CMMRD.	Strong
<b>Rec. 2</b>	Psychological support should be offered to patients with CMMRD and their families at any time during treatment and cancer surveillance.	Strong
<b>Rec. 3</b>	Age adapted education about CMMRD should probably be offered to CMMRD patients and their families.	Strong

<b>Rec. 4</b>	Healthcare professionals involved in diagnosis and surveillance should address the psychosocial implications of a diagnosis of CMMRD.	Strong
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## 9.5 CLINICAL MANAGEMENT - SUMMARY OF EVIDENCE AND GUIDELINE RECOMMENDATIONS

Data allowing assessment of the efficacy of standard management for CMMRD-associated tumours are still limited. Given the involvement of the MMR pathway in the induction of cell death after DNA damage (Gupta & Heinen, 2019; Mas-Ponte et al., 2022), there are concerns about the risk of resistance of CMMRD-associated tumours to several types of genotoxic agents. However, clinical data comparing response to treatment in CMMRD-associated tumours with those reported in sporadic tumours are still lacking.

### Chemotherapy

No systematic studies evaluating the efficacy and toxicity of radiotherapy or chemotherapy have been conducted in CMMRD patients. There are pre-clinical data suggesting tolerance of CMMRD-associated tumours to several types of chemotherapy such as thiopurines and methylating agents, which rely on a functional MMR system to be effective. (Aquilina et al., 1990; Bignami et al., 2003; Gupta & Heinen, 2019; Karran et al., 2003; Mas-Ponte et al., 2022; Stojic et al., 2004). Furthermore, the resistance of EBV-transformed lymphocytes of CMMRD patients to the methylating agent temozolomide has been extensively studied and can be used as an ancillary assay to confirm or refute a CMMRD diagnosis if genetic testing is inconclusive (see 9.1 Rec. 12 and Tables 3 and 4) (Bodo et al., 2015). Data concerning other drugs are more controversial. Pre-clinical data suggest that a functional MMR pathway is essential to maintain the sensitivity to several drugs such as cisplatin (Sawant et al., 2015; Stojic et al., 2004) or 5-fluorouracil (5-FU) (Meyers et al., 2001). However, meta-analysis of clinical trials performed in Lynch syndrome do not rule out the efficacy of 5 FU or platinum-based treatment in patients with germline MMR PVs and, hence, MMR deficient tumours (Tomasello et al., 2022).



Several authors have suggested that chemotherapy may increase the risk of second malignancies in CMMRD by increasing the rate of unrepaired somatic mutations (Karran et al., 2003), but we are still lacking studies allowing evaluation of the role of chemotherapy in the pathogenesis of second malignancies in CMMRD patients.

### **Immunotherapy**

Due to the role of the MMR system in replication error repair, most tumours of patients with CMMRD are hypermutated and can even be ultramutated due to a combined constitutional MMR and somatic polymerase proofreading defect (Andrianova et al., 2017; Shlien et al., 2015; Waterfall & Meltzer, 2015). These mutations lead to the formation of tumour-specific neoantigens, which may serve as targets for the immune system and thus, MMR deficient tumours are more likely to respond to immune checkpoint blockade therapy (reviewed by (Michaeli & Tabori, 2018; Westdorp et al., 2017).

There is growing evidence of the efficacy of immune checkpoint inhibitors (ICI) in cancer patients with an impaired MMR system such as Lynch syndrome or CMMRD patients (Bouffet et al., 2016; Therkildsen et al., 2021). The efficacy of ICIs in the treatment of metastatic MSI colorectal cancer was first demonstrated in 2015 (Andre et al., 2020; Le et al., 2017; Le et al., 2015). Since then, other studies suggest an important role of ICI in the adjuvant and neoadjuvant setting (Cercek et al., 2022). In 2017 Pembrolizumab and Nivolumab were approved by the Food and Drug Administration (FDA) for the treatment of MMR-deficient cancers, regardless of tumour site or histology (reviewed by Quinn et al. (Quinn & Nichols, 2017) and Michaeli et al. (Michaeli & Tabori, 2018)). Immunotherapy should be discussed for the treatment of any tumour arising in the context of CMMRD, especially tumours of the Lynch syndrome-spectrum, from the initial phase of management to management for advanced disease.

Several durable responses after ICI in diverse tumour types have been described in CMMRD patients (AlHarbi et al., 2018; Bouffet et al., 2016; Mishra et al., 2022; Paul et al., 2020; Pavelka et al., 2019; Rittberg et al., 2021; Westdorp et al., 2017; Xie et al., 2021). The

C4MMRD consortium reported that among a series of 18 CMMRD patients treated with ICI (13 with HGG, four with digestive tract tumours and four with NHL including three patients with both NHL and another tumour), eight (44%) benefited from the treatment with a durable response and/or a stabilization (Suerink et al., 2021a).

More recently, the IRRDC reported a series of 38 patients with CMMRD (n=28), Lynch syndrome (n=8) or polymerase proofreading deficiency (n=2) treated with an ICI. A response or a stable disease was observed in 55.5% of the tumours. The response was sustained in 80% of the cases at last follow-up (median duration 1.87 years). The 3-year survival was 41.4% (Das et al., 2022). Response rate was 64% in brain tumours (n=31) and 100% in the non-CNS solid tumours (n=11) whereas none of the three cases with lymphoma or leukaemia experienced a response. Response to ICI was associated with ultra-high mutation burden (>100 mutations per Mb) in patients with combined MMR and polymerase proofreading deficiency. Also, in tumours with a mutation burden <100 mutations per Mb, response was associated with the burden of microsatellite insertions and deletions. Importantly, pseudo-progressions linked to an acute reaction to immunotherapy (a tumour flare reaction) were reported in 27% of the patients in this series. Distinguishing these flare effects from a true tumour progression is crucial since durable responses were observed in the patients who continued treatment with ICI after flare reaction (Das et al., 2022). Furthermore, the genomic and immune profile of tumours experiencing flare was similar to those of patients who experienced a response suggesting that the patients who experience a flare effect are likely to be responders.

Additionally, Bouffet et al. reported that low-grade tumours of CMMRD patients, including low-grade gliomas, are not (ultra-)hypermutated and, therefore, are not expected to respond to PD-1 blockade (Bouffet et al., 2016).

## **Specific considerations for management of brain tumours**

### **High-grade gliomas (HGGs)**

To date, surgery is still the mainstay for resectable HGG. There is some data suggesting that neo-adjuvant administration of ICI could improve the prognosis of high- grade glioma

outside the CMMRD spectrum (Cloughesy et al., 2019). Given the high response rate to ICI in patients with CMMRD, including ICI in the front-line treatment of patients with HGG should be an option whenever possible (Rec. 7).

CMMRD syndrome is not associated with increased radiosensitivity. No side effects have been described in patients who received both ICI and radiotherapy with large radiation fields (Sahebjam et al., 2021). Therefore, there is currently no evidence to support a contraindication to offering concomitant immunotherapy to patients treated with broad irradiation fields (including craniospinal irradiation) (Rec. 4).

Preclinical data highlight the risk of resistance of MMR-deficient HGGs to temozolomide (Abidi et al., 2021; Carrato et al., 2021; Johannesma et al., 2011; Kebudi et al., 2020; Pollack et al., 2010; Ripperger et al., 2010; Scott et al., 2007; Touat et al., 2020; Westdorp et al., 2017). Moreover, it has been shown that recurrent glioblastomas after temozolomide frequently exhibit a hypermutated phenotype with defective MMR (Touat, Nature 2020) confirming previous preclinical evidence that MMR defects are a major mechanism of resistance to temozolomide (Gan et al., 2022). Taken together, temozolomide is no longer recommended in CMMRD patients despite the lack of a study comparing the efficacy of temozolomide in a series of patients with sporadic and CMMRD-associated HGG (Rec. 6).

Data about natural history of low-grade glioma in CMMRD were provided by Durno et al. through reporting the experience of brain surveillance MRI in CMMRD patients. All six non-resected low-grade gliomas underwent transformation into malignant HGG in a median of 1.7 years (Durno et al., 2021). Therefore, resection, whenever possible, without excessive neurological risks, is clearly advisable (Rec. 8).

No evidence for a specific treatment of CMMRD-associated medulloblastoma could be found in the literature. Currently, it should probably not differ from treatment of sporadic medulloblastoma, except for the use of temozolomide as maintenance or second-line therapy, which is not recommended in CMMRD (Rec. 6, 9).

### **Specific considerations for management of haematological malignancies**

## Haematological malignancies

Evidence for best treatment of haematological malignancies in CMMRD patients is still limited. The reduced biologic activity of thiopurines in MMR deficient cells has been suspected to be associated with a lower efficacy of treatment in this population and to be involved in the genesis of second malignancies (Kroeze et al., 2022; Lavoine et al., 2015; Ripperger et al., 2021). Kroeze et al. (2022) indirectly demonstrate the low biological effect of 6-mercaptopurine in vivo by showing that the doses of Purinethol had to be increased at the maximum level (200% of starting dose) without inducing any haematotoxicity during maintenance in four patients with a lymphoblastic lymphoma (Kroeze et al., 2022).

Several publications on small series of CMMRD-associated NHL reported lower survival when compared to sporadic lymphomas due to an excess of second malignancies but also of a slight excess of progressions/relapses mainly in T cell lymphoblastic lymphomas. B cell lymphomas are rarer and seem not to be associated with an excess of treatment failures (Kroeze et al., 2022; Lavoine et al., 2015; Ripperger & Schlegelberger, 2016). In the collaborative study performed by the European C4CMMRD Consortium, the IRRDC in Canada and the European Intergroup for childhood non-Hodgkin Lymphoma (EICNHL), which reviewed 100 CMMRD-associated NHL in 74 patients, the 3-year cumulative risk of progression/relapse after a first lymphoma treated with current chemotherapy regimen was 20.8%. This rate is considered acceptable in a series of patients treated over a long period of time and, for a third of them, after a previous malignancy. However, the risk of second malignancies including multiple NHL was very high in these patients leading to poor overall survival (Rigaud et al., 2024). The role of genotoxic chemotherapy in the genesis of these second malignancies could not be assessed. Considering that response to current chemotherapy regimen in CMMRD-associated NHL is similar to that in sporadic NHL, and as there is no evidence showing chemotherapy's contribution to the development of secondary malignancies, nor any alternative treatment that has proven to be equally effective, the treatment approach for CMMRD NHL should probably be similar to the current standard regimens used for non-CMMRD cases (Rec. 10). In the recent collaborative study on CMMRD-associated NHL, all 20 second lymphomas were treated with curative intent with most

(15/20) following standard regimens designed for initial treatment of each NHL subtype. As only 3/20 patients experienced a failure (one toxicity-related death, one early progression and one relapse), this strategy is probably suitable for second NHL (Rec. 11). As in all second malignancies, the cumulative doses of each drug already received by the patient should be taken into account in the decision-making process.

No study comparing the outcome of CMMRD-associated leukaemias with sporadic leukaemias of the same subtype has been performed so far. Currently, treatment should probably not differ from treatment of sporadic leukaemias (Rec. 12).

Haematopoietic stem cell transplantation seems feasible. In their literature review from 2016, Ripperger and Schlegelberger (Ripperger & Schlegelberger, 2016) reported five patients treated with haematopoietic stem cell transplantation with no major side effects except for a post-transplant lymphoproliferative disorder in one patient (Elhasid et al., 2015) (Rec. 5).

### **Colorectal cancers and polyps**

Recommendations for colorectal cancer management are well established for patients with Lynch syndrome (Ozyoruk et al., 2021), but very few data are available for CMMRD patients. The European Consortium C<sub>4</sub>CMMRD and the US Multi-Society Task Force on Colorectal Cancer, agree on the recommendation of extensive gastrointestinal surgery as the treatment of choice in CMMRD patients with colorectal cancer. However, the balance of benefits and risks of extensive surgery for treatment of CMMRD colorectal cancer is unclear (Levi et al., 2015). Extensive polyposis of the colon is not frequent (median number of adenoma per patient is 12 in the European C<sub>4</sub>CMMRD database-personal data C. Colas) so most of the time polyps may be managed by endoscopic resection (van Leerdam et al., 2019). Surgical decisions must always take into account individual situations, in particular other tumour histories, and be discussed by a specialised board. In patients with colonic adenomas containing high-grade dysplasia or cancer, or when there are too many polyps to remove endoscopically, total or subtotal colectomy with ileorectal anastomosis should be discussed on a case-by-case basis as in other polyposis syndromes (Rec. 17). In the case of rectal cancer,

a proctocolectomy with ileal pouch-anal anastomosis may be necessary (Durno et al., 2017a; Tabori et al., 2017; Vasen et al., 2014).

The impact of a deficient MMR pathway on the efficacy of use of 5-FU is still a matter of debate (Aggarwal et al., 2022). While preclinical data and several trials lead to consider MMR deficient colorectal cancers as having a degree of tolerance for 5-FU (Carethers et al., 1999) this drug is not contra-indicated in patients with Lynch syndrome (Tomasello et al., 2022). However, ICIs have shown their efficacy in this context and now represent the standard of care for metastatic and advanced colorectal cancer patients with Lynch syndrome (Jin & Sinicrope, 2022). Therefore, it is recommended that management of cancers of the Lynch syndrome-spectrum in a CMMRD patient follow treatment guidelines designed for patients with Lynch syndrome associated tumours, and that immunotherapy should be used as front-line treatment of large, unresectable or metastatic colorectal tumours in a CMMRD patient (Rec. 13-14).

In addition, several case reports suggest a potential preventive effect of immunotherapy on polyposis development. In one CMMRD case, it was reported that colonic adenomas gradually decreased in number and size during treatment of a medulloblastoma with anti-PD1, but new polyps appeared one year after the end of the anti-PD1 treatment (Ozyoruk et al., 2021). Another patient received pembrolizumab (200 mg/course) at 3 week intervals for 6 months as neoadjuvant therapy for gastric cancer and a clinical improvement of the polyposis phenotype was observed, together with no immune-related adverse events (Tanimura et al., 2022).

### **Other Lynch syndrome-associated tumours: ovarian, endometrium and gastric tumours**

There is no specific evidence in literature for the treatment of these tumours in CMMRD patients. Their management should probably not differ from those in a LS context, highlighting the role of immunotherapy (Rec. 13, 15).

### **Specific considerations for management of other tumours**

Other tumours are rare in CMMRD accounting for less than 10% of the cases. Several tumour types have been described including sarcomas, neuroblastomas, neuroblastomas and retinoblastomas. Although there is no direct evidence of the efficacy of ICIs in these tumour types in CMMRD patients, ICIs may be effective in MMR deficient tumours across a variety of different tumour types (Le et al., 2017) (Rec. 16).

### **Multiple tumours**

Considering the high incidence of multiple tumours in CMMRD patients, we recommend separate sampling and molecular analysis of synchronous tumours as they might be distinct entities. Cancer surveillance should be performed at the time of diagnosis as well as during and after the period of cancer treatment according to the guidelines (rec. 18).

In this context, in case of suspicion of a relapse, it is crucial to consider the possibility of a second primary disease rather than a relapse and to perform molecular analysis of samples at initial diagnosis and relapse in order to be able to make an accurate diagnosis (rec. 19-20)

### **Biobanking**

There are several aspects of CMMRD-associated tumours that require further investigation (predictive biomarkers for immunotherapy response, the impact of chemotherapy on the development of multiple malignancies, and the underlying mechanisms of progression from low-grade to malignant tumours; see section 11 - Future Research). Collecting and preserving fresh tumour specimens is crucial due to the rarity of this condition. Additionally, access to frozen material from previous tumours is vital for patient care, especially when comparing genomic analyses of two sequential tumours to differentiate between relapse and the development of a second malignancy (rec. 19-21).

### **Prevention of colorectal cancer with low-dose acetylsalicylic acid**

Several studies performed in patients with Lynch syndrome have shown a significant reduction of colorectal cancer risk associated with prolonged administration of low dose acetylsalicylic acid (reviewed by Serrano et al. (Serrano et al., 2022)). The CAPP2 trial compared a 2-y treatment with aspirin at 600 mg to placebo in patients with Lynch syndrome

and demonstrated that acetylsalicylic acid significantly decreased the risk of colorectal cancer at 10-years of follow-up (adjusted HR = 0.65, 95% CI [0.43–0.97]) without increasing the risk of serious adverse events (Burn et al., 2020). However, no difference was seen concerning the risk of other cancers of the Lynch syndrome spectrum (adjusted HR = 0.94, 95% CI [0.59–1.50]). International guidelines suggest discussing with Lynch syndrome carriers the possibility of using low-dose acetylsalicylic acid for colorectal cancer prevention.

Data on acetylsalicylic acid as a preventive drug for colorectal cancer in CMMRD are very limited. As the risk of tumours is much higher in CMMRD than in Lynch syndrome, the beneficial effect of acetylsalicylic acid in CMMRD patients may be different and still has to be evaluated (Durno et al., 2017a). Additionally, the potential benefit has to be balanced with the theoretical haemorrhagic risk in brain tumours and developmental venous anomalies (Guerrini-Rousseau et al., 2019). Leenders et al. (2018) reported the case of a *PMS2* CMMRD patient who took acetylsalicylic acid during 5.5 years with no adverse event and had not developed gastrointestinal lesions at 16 years of age (Leenders et al., 2018). Two other patients have been reported but with a short follow-up (Biller et al., 2016; Ramchander et al., 2017). The short follow-up data available and unpublished anecdotal cases of patients who have continued to develop new polyps under long-term acetylsalicylic acid intake do not allow any conclusion about the possible efficacy of acetylsalicylic acid in CMMRD. However, the potential benefits and side effects of preventive treatment with acetylsalicylic acid should probably be discussed with CMMRD patients (Rec. 22).

### **Immunoglobulins defects**

The MMR system is involved in immunoglobulin class-switch recombination and in somatic hypermutation. Both processes are needed for B cell maturation and for diversification and specification of the mammalian immunoglobulin repertoire. Although IgG2/4 subclass deficiency, IgA deficiency, or - rarely - more severe phenotypes of antibody formation, B cell class switch, maturation, and memory formation defects may be found in patients with CMMRD, they are neither constant nor obligatory diagnostic hallmarks of this syndrome and tend to lack a clinical correlate (Tesch et al., 2018). Hence, CMMRD patients should not be



treated to compensate for the inherent deficit in the absence of clinical manifestations (Rec 23).

Recommendations		Strength
<b>Rec. 1</b>	Multiple patients with CMMRD have been cured from a cancer diagnosis. Thus, in a CMMRD patient diagnosed with cancer, a curative approach should be considered and evaluated.	Strong
<b>Rec. 2</b>	For several cancer types, no CMMRD specific treatment recommendations exist. Treatment of patients with CMMRD related neoplasms should, therefore, probably be discussed in a multidisciplinary board with a treating physician, an expert for the patient's cancer type as well as a CMMRD expert.	Strong
<b>Rec. 3</b>	Patients with CMMRD associated neoplasms should probably be included in clinical trials whenever possible.	Strong
<b>Rec. 4</b>	CMMRD is probably not a contraindication for radiotherapy, if indicated.	Moderate
<b>Rec. 5</b>	CMMRD is probably not a contraindication for haematopoietic stem cell transplantation, if indicated.	Moderate
<b>Rec. 6</b>	Temozolomide should probably be avoided in patients with CMMRD-associated high-grade glioma.	Strong
<b>Rec. 7</b>	The use of immunotherapy with a PD1 inhibitor should be considered for CMMRD patients with high-grade glioma, preferentially within a clinical trial.	Strong
<b>Rec. 8</b>	CMMRD-associated low grade glioma should probably be resected whenever possible without excessive neurological risks.	Strong

<b>Rec. 9</b>	Front-line treatment of CMMRD-associated medulloblastoma should probably not differ from treatment of sporadic medulloblastoma/primitive neuro-ectodermal tumours.	Moderate
<b>Rec. 10</b>	In case of CMMRD-associated non-Hodgkin lymphoma, chemotherapy should probably be similar to the treatment of the same tumour without CMMRD.	Moderate
<b>Rec. 11</b>	In case of a second primary non-Hodgkin lymphoma in a CMMRD patient, standard first-line treatment adapted to the non-Hodgkin lymphoma subtype taking into account cumulative doses of chemotherapy previously received should probably be given rather than a relapse treatment.	Moderate
<b>Rec. 12</b>	In case of CMMRD-associated leukaemia, chemotherapy should probably be similar to the treatment of the same cancer without CMMRD.	Moderate
<b>Rec. 13</b>	In case of diagnosis of a cancer of the Lynch spectrum in a CMMRD patient, treatment guidelines designed for patients with Lynch syndrome associated tumours should be followed.	Strong
<b>Rec. 14</b>	Immunotherapy should be recommended as front-line treatment of large, unresectable or metastatic colorectal tumours in a CMMRD patient	Strong
<b>Rec. 15</b>	Immunotherapy should be performed front-line for all extra-colorectal Lynch-related tumours in CMMRD patients ideally in therapeutic trials.	Strong
<b>Rec. 16</b>	Immunotherapy should be discussed and encouraged within an expert centre for any non-Lynch related tumour at any time during treatment (diagnosis or relapse) of a CMMRD patient, especially if standard therapeutic guidelines offer only low chance of cure.	Moderate

<b>Rec. 17</b>	CMMRD patients with multiple colonic adenomas should probably be surgically managed according to guidelines developed for other polyposis syndromes.	Strong
<b>Rec. 18</b>	CMMRD patients may present with multiple tumours at the same time or may develop additional tumours during treatment. Thus, cancer surveillance around the time of diagnosis and during the period of cancer treatment should be offered.	Strong
<b>Rec. 19</b>	In CMMRD patients with a suspected relapse, a second primary disease should be considered. This may influence the treatment choice.	Strong
<b>Rec. 20</b>	In case of relapse of a CMMRD-associated tumour, molecular analysis of samples at initial diagnosis and relapse should be performed to differentiate a relapse from a second primary tumour.	Strong
<b>Rec. 21</b>	Fresh tumour specimens should be collected and stored (or directly molecularly analysed) whenever possible and if the CMMRD patient and/or their family approves. This may be relevant for research as well as for clinical purposes (e.g. see Rec 19).	Strong
<b>Rec. 22</b>	Advantages and potential side effects of preventive treatment with acetylsalicylic acid should probably be discussed with CMMRD patients.	Moderate
<b>Rec. 23</b>	CMMRD patients with IgG/A reduced levels/deficiency should not be treated to compensate for the inherent deficit in the absence of clinical manifestations.	Strong

## 10. WHAT DO OTHER GUIDELINES STATE?

These are the first comprehensive guidelines addressing the most important aspects of care for CMMRD consisting of 82 recommendations across five sections: *diagnosis, genetic counselling, surveillance, quality of life, and clinical management*. Previous guidelines focused only on one or two of these topics, mainly diagnosis and surveillance, and mentioned other aspects of CMMRD care, such as clinical management, genetic counselling and quality of life, only in their discussions. In the following, we compare the recommendations given here with recommendations in existing guidelines for the diagnosis and surveillance of CMMRD only due to a lack of previous guidelines on genetic counselling, quality of life, and clinical management.

Section 7.1 on the diagnosis of CMMRD includes three subsections. The first subsection includes five recommendations (Rec 1, 2, 3, 4 and 6) on which children/young adults should be tested for CMMRD. Previous guidelines addressing this question come from the C<sub>4</sub>CMMRD consortium (Suerink et al., 2018; Wimmer et al., 2014), from an international consensus working group consisting of IRRDC and C<sub>4</sub>CMMRD members (Aronson et al., 2022), and from the US Multi-Society Task Force on Colorectal Cancer (Durno et al., 2017a). The recommendations 1 and 6 are based on C<sub>4</sub>CMMRD guidelines for the clinical indication of diagnostic CMMRD testing in cancer patients (Wimmer et al., 2014, Table 1), and in children suspected to have sporadic NF1/LGSS without cancer and without an *NF1/SPRED1* germline (L)PV after comprehensive genetic analysis (Suerink et al., 2018, Table 2). While the latter guidelines are included here without any change, the guidelines on the clinical indication for CMMRD testing in cancer patients have been adapted to include novel findings (for details see 9.1). The original C<sub>4</sub>CMMRD guidelines (Wimmer et al., 2014) are widely used and were included also as a diagnostic entry point in recommendations from the international consensus working group, consisting of members of the IRRDC and C<sub>4</sub>CMMRD (Aronson et al., 2022) and the Pediatric Cancer Working Group of the AACR (Tabori et al., 2017). In a consensus statement of the US Multi-Society Task Force on Colorectal Cancer, in addition to clinical features covered by the C<sub>4</sub>CMMRD guidelines for cancer patients, a tumour with a high TMB (Rec. 2) or expression loss of one or more of the four MMR proteins in neoplastic and in non-neoplastic cells (Rec. 3) are recommended criteria for CMMRD

testing (Durno et al., 2017b). The identification of a heterozygous (likely) pathogenic variant in one of the MMR genes in a cancer patient aged <18 years (Rec. 4) has not been defined in any previous guidelines as an indication for CMMRD testing. One study suggested testing all malignant brain tumours for expression loss of one or more MMR genes to screen for CMMRD (Carrato et al., 2021). However, we did not include this recommendation in these guidelines as the clinical utility of this approach needs further exploration.

In the second and third subsections of the diagnosis section 7.1, we have formulated an optimal CMMRD testing strategy (Rec. 7-11) and criteria for a definitive CMMRD diagnosis (Rec. 12-14) across eight recommendations. Some aspects of these have been discussed in previous guidelines (Suerink et al., 2018; Wimmer et al., 2014) and/or formulated into the diagnostic criteria provided in the international consensus working group recommendations (Aronson et al., 2022). However, there are several differences in the present recommendations compared to these earlier guidelines. Here, Table 4 lists ancillary tests that can confirm or refute CMMRD if genetic testing is inconclusive. In contrast to the previous recommendations (Aronson et al., 2022), IHC of the four MMR proteins is not included as an ancillary test in this list, as this approach can generate both false positive and false negative results and, therefore, is not suitable to confirm or refute CMMRD. Similarly, the in vitro repair assay developed by Shuen et al (Shuen et al., 2019) is not listed as an ancillary test, because we are not aware of its use in Europe. To our knowledge it has not been evaluated in a validation cohort that is independent from the cohort of 20 confirmed CMMRD cases used to set the diagnostic threshold of 10% repair activity. All ancillary assays listed in Table 4 have been evaluated in large cohorts of positive and negative controls. With the exception of the gMSI assay (Ingham et al., 2013), which is insensitive to MSH6-associated CMMRD, all have achieved 100% specificity and sensitivity (Bodo et al., 2015; Chung et al., 2023; Gallon et al., 2023; Gonzalez-Acosta et al., 2020). A positive result from any of these single ancillary tests (including the gMSI assay) can confirm a CMMRD diagnosis when genetic testing is inconclusive, provided that the assay has been thoroughly evaluated in the laboratory. This is in contrast to the diagnostic criteria of the international consensus working group (Aronson et al., 2022). For cases lacking homozygosity or compound heterozygosity of MMR variants classified as pathogenic (class 5 according to the [ClinGen](#)

[InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel](#)), the international consensus working group defined “hallmark cancers” of CMMRD and recommended distinct diagnostic criteria with respect to ancillary assay test results between cases in whom a hallmark cancer is present or absent. In absence of a hallmark cancer, two ancillary assays need to be positive to confirm a CMMRD diagnosis, whereas in presence of a hallmark cancer, one positive ancillary assay is sufficient. The concept of hallmark cancers determining likelihood of a CMMRD diagnosis and, therefore, dictating criteria for diagnosis by ancillary tests is not adopted by these guidelines. However, the adaptations of C<sub>4</sub>CMMRD indication criteria for CMMRD testing in cancer patients (Table 2, see also 9.1) have become more stringent and, likely, more specific. Therefore, patients selected for CMMRD testing will have a higher probability of having CMMRD when compared to the original C<sub>4</sub>CMMRD criteria (Wimmer et al., 2014). Furthermore, the present recommendations provide more comprehensive guidance for the diagnosis of patients in whom only one or no MMR variant classified as (L)PV or VUS is identified. In such cases, transcript analysis should show either faulty splicing or reduced expression of the wild-type allele(s) in addition to a positive ancillary test result for a CMMRD diagnosis to be made (Rec. 12, Table 5). Transcript analysis is not included in the international consensus working group recommendations (Aronson et al., 2022). Another difference between the diagnostic criteria of these guidelines and of those of the international consensus working group is that the present criteria make no distinction between a definite and a likely diagnosis.

Previous surveillance protocols for CMMRD patients were provided by the C<sub>4</sub>CMMRD consortium (Vasen et al., 2014), the Pediatric Cancer Working Group of the AACR (Tabori et al., 2017) and the US Multi-Society Task Force on Colorectal Cancer (Durno et al., 2017b). Table 6 compares these previous recommendations with those given in section 7.3.

Sections 7.2, 7.4 and 7.5 contain recommendations regarding the genetic counselling, the quality of life and the clinical management of CMMRD patients. Recommendations addressing these topics have been formulated for the first time in these guidelines. For previous publications that provide the evidence underpinning these recommendations, see sections 9.2, 9.4 and 9.5.

**Table 6: Comparison of cancer surveillance protocols for CMMRD patients**

	ERN GENTURIS Guidelines	C4CMMRD Guidelines (Vasen et al. 2014)	Pediatric Cancer working group of the AACR (Tabori et al. 2017)	US Multi-Society Task Force on colorectal cancer (Durno et al. 2017)
<b>Education of and communication with CMMRD patients and/or their parent(s)</b>				
Tumour risk	educate about tumour risks associated with CMMRD	no recommendation	no recommendation	no recommendation
Tumor related symptoms	educate about symptoms related to the main tumours	advice to contact doctor in case of unusual signs or symptoms	educate about symptoms of tumours causing abdominal masses and haematologic malignancies	no recommendation
Surveillance	discuss pros and cons to make a joint decision with the clinician on surveillance program participation	best approach might be to discuss pros and cons to make a joint decision with the clinician on surveillance program participation	no recommendation	no recommendation
Research participation	probably encourage to communicate their screening results in research projects or databases	no recommendation	no recommendation	no recommendation
<b>Clinical examination to screen for all tumours</b>				
Age to start	not specified	1 year	no recommendation	no recommendation
Periodicity	1x/6 months	1x/6 months	n.a.	n.a.
Modality	not specified	not specified	n.a.	n.a.
<b>Whole body MRI to screen for all tumours</b>				
Age to start	probably at CMMRD diagnosis or when anaesthesia is no longer required	no recommendation (a prospective randomised trial needed to test efficacy of WBMRI 1x/year)	6 years	uncertain (rapid WBMRI might be considered)
Periodicity	probably at least once	n.a.	1x/year	n.a.
	probably discuss with patient and/or their parents annual as an screening option with weak evidence of efficacy			
Modality	not specified	n.a.	should not replace brain MRI	n.a.
<b>Brain MRI to screen for brain tumors</b>				

Age to start	probably at CMMRD diagnosis or at least 2 years	2 years	at CMMRD diagnosis	2 years
Periodicity	1x/6 months at age <20 years, at least 1x/year at age ≥20 years	1x/6-12 months	1x/6 months	1x/6 months
Modality	first brain MRI probably with contrast enhancement	not specified	should not be replaced with WB-MRI	optional head ultrasound starting at 6 months until fusion of fontanelle
	in patients without a previous brain tumour, probably include anatomical sequence T2 FLAIR (if possible in 3D) combined with MRI diffusion sequence			
	in patients with a previous brain tumour, include anatomical sequences T2-FLAIR, diffusion sequence, and T1+ contrast enhancement (if possible in 3D)			
<b>Abdominal ultrasound and blood counts to screen for abdominal lymphomas and other haematological (pre-)malignancies</b>				
Age to start	probably neither should be performed	1 year	birth (blood count) 1 year (abdominal ultrasound)	1 year
Periodicity	n.a.	1x/6 months	1x/6 months	1x/6 months
Modality	n.a.	blood count, optional abdominal ultrasound	optional blood count, optional abdominal ultrasound	blood count only
<b>(Ileo)colonoscopy to screen for colorectal cancer and terminal ileal cancer</b>				
Age to start	probably 6 years	8 years	4-6 years	6 years
Periodicity	1x/year (do not exceed 1 year interval)	1x/year	1x/year	1x/year
	probably increase frequency up to 6 months-interval once polyps are detected	increase frequency to 1x/6 months interval once polyps are detected	increase frequency once polyps are detected	increase frequency to 1x/6 months interval once polyps are detected
Modality	probably perform with coloration	use chromoscopy	not specified	not specified
	probably perform in a centre with gastroenterologists experienced in Lynch syndrome screening			
<b>Upper gastrointestinal endoscopy to screen for oesophageal, gastric, and duodenal cancer</b>				



Age to start	probably same as colonoscopy or at least 10 years	10 years	4-6 years	8 years
Periodicity	1x/year (do not exceed 1 year interval)	1x/year	1x/year	1x/year
	probably increase frequency up to 6 months-interval once polyps are detected		increase frequency once polyps are detected	
Modality	probably use push enteroscopy and careful inspect of the ampullary region	can be done at the same time as colonoscopy under general anaesthesia	not specified	not specified
	probably perform with coloration			
	probably perform in a centre with gastroenterologists experienced in Lynch syndrome screening			
Video capsule endoscopy to screen for gastrointestinal cancer				
Age to start	probably 10 years	10 years	4-6 years	8 years
Periodicity	1x/year	1x/year	1x/year	1x/year
Modality	not specified	not specified	not specified	not specified
Monitoring of haemoglobin levels to screen for gastrointestinal cancers				
Age to start	no recommendation	no recommendation	no recommendation	8 years
Periodicity	n.a.	n.a.	n.a.	1x/6 months
Modality	n.a.	n.a.	n.a.	not specified
Clinical examination and transvaginal ultrasound to screen for gynaecological cancer				
Age to start	20 years	20 years	20 years	20 years
Periodicity	probably 1x/year	1x/year	1x/year	1x/year
Modality	not specified	not specified	not specified	not specified
Pipelle or curettage to screen for gynaecological cancer				
Age to start	no recommendation	20 years	20 years	20 years
Periodicity	n.a.	1x/year	1x/year	1x/year
Modality	n.a.	not specified	not specified	not specified
Abdominopelvic ultrasound to screen for gynaecological and urinary tract cancer				
Age to start	20 years	no recommendation	no recommendation	no recommendation
Periodicity	probably 1x/year	n.a.	n.a.	n.a.
Modality	not specified	n.a.	n.a.	n.a.
Annual urine cytology and urine dipstick to screen for urinary tract cancer				
Age to start	probably do not offer	20 years	20 years	10 years
Periodicity	n.a.	1x/year	1x/year	1x/year
Modality	n.a.	not specified	not specified	alternative MRI to be considered
Breast cancer screening				

	probably follow general population guidelines	no recommendation	no recommendation	no recommendation
Offer specific surveillance of low-grade lesions				
	recommended	no recommendation	no recommendation	no recommendation

## 11. SUGGESTIONS FOR FUTURE RESEARCH

CMMRD has a birth incidence of approximately one in a million based on the estimated frequency of Lynch syndrome carriers in the general population and the chance that parents carrying PVs in the same MMR gene will have a child inheriting both PVs (Suerink et al., 2019a). Being so rare, characterisation of CMMRD pathology and clinical course is difficult and requires concerted, international effort. Therefore, a general priority for future research is to maintain and expand the clinical and academic networks around CMMRD. Patient databases are of particular importance, allowing better description of the syndrome and prospective follow up of patients that will support all aspects of future research. Currently, there are two major databases maintained by the IRRDC and European C<sub>4</sub>CMMRD consortium, but many countries have few or no patients listed within these or within databases of their own. Therefore, a sustained effort to collate patient data and samples is needed. Similarly, biobanking of CMMRD neoplastic and non-neoplastic tissues would provide a resource for a variety of studies into CMMRD-related tumourigenesis that are currently not possible. Following are more specific suggestions for future research.

The estimations of the population frequency of CMMRD have not been confirmed empirically, and studies dedicated to the detection of CMMRD among different patient populations are lacking. The few studies that can provide empirical evidence typically report diagnoses from (L)PV only, meaning potential CMMRD cases whose MMR variants were not detected or were considered VUS were not reported (Attarbaschi et al., 2021; de Voer et al., 2021; Supp. Tables S1 & S7 in Gröbner et al., 2018; Kroeze et al., 2022). Therefore, there is some uncertainty in the frequency of CMMRD in different patient groups and studies designed to specifically address this could be informative for future testing guidelines. Prospective screening for CMMRD in relevant patient cohorts (paediatric high-grade glioma or T-lymphoblastic lymphoma patients, children suspected of sporadic NF1, etc.) using scalable, highly reliable, and low-cost ancillary assays, should improve frequency estimation which will have an impact on future guidelines for CMMRD diagnosis. Such studies would also lay the basis for an evaluation of the sensitivity and specificity of the clinical indication criteria for CMMRD in cancer patients (Table 2) to assess their efficacy and potential weaknesses for refinement. Several relatively low cost, high sensitivity, and high specificity methods are now available that could be used for molecular screening for CMMRD. These

utilise next generation sequencing to detect MSI in non-neoplastic peripheral blood leukocytes as a diagnostic hallmark of CMMRD. These include amplicon sequencing of select MSI markers (Gallon et al., 2019; Gallon et al., 2023; Gonzalez-Acosta et al., 2020; Marin et al., 2024) and the Low-pass Genomic Instability Characterization (LOGIC) assay that uses low depth whole genome sequencing (Chung et al., 2023). Although associated with more limitations, tumour-based molecular screening for CMMRD may also be useful. For example, screening by IHC analysis of MMR protein expression in tumour and normal cells (Carrato et al., 2021) and analyses of tumour mutation burden and mutational signatures (Gröbner et al., 2018; Thatikonda et al., 2023) could be used to identify patients in relevant patient cohorts. Another advantage of these ancillary assays is that they can be used to interpret the pathogenicity of VUS or can detect CMMRD diagnoses missed by routine genetic analysis. For example, structural variants disrupting MMR genes or variants in exons 12-15 of *PMS2* can be difficult to detect by conventional short read sequencing technologies.

CMMRD cases are often detected by testing of patients fulfilling specific clinical characteristics, including age of cancer diagnosis and type of cancer, which likely biases our understanding of the CMMRD phenotype. In Lynch syndrome, it has been shown through decades of research that ascertainment bias initially led to a significant over-estimation of cancer risk. Early reports based predominantly on probands and high risk families suggested an average age of CRC diagnosis of 44 years, whereas risk estimation using Lynch syndrome carriers ascertained both from high risk families and through molecular testing (for example, cascade testing of unaffected relatives) found a much later average age of onset of 61 years (Hampel et al., 2005). Through extensive characterisation of affected families, it also became apparent that extracolonic manifestations of Lynch syndrome are a significant part of their tumour burden (Watson et al., 2008). These observations of a lower penetrance and broader cancer spectrum compared to early studies have been confirmed by the Prospective Lynch Syndrome Database study that includes prospective data from over 6000 Lynch syndrome gene carriers (Dominguez-Valentin et al., 2020; Moller et al., 2017). Similarly, it is probable that the CMMRD phenotype is overall less severe and may be more diverse than we currently estimate, and this may have implications for future diagnosis and management guidelines. Molecular screening for Lynch syndrome has significantly impacted our understanding of its frequency and phenotype by reducing clinical ascertainment bias. Strategies include

universal testing of colorectal and endometrial cancers for loss of MMR function either through IHC, MSI analysis, or tumour sequencing to select patients for germline genetic analysis, as well as immediate germline testing in cases with personal or family history indicative of Lynch syndrome. Molecular screening for CMMRD in relevant patient cohorts could also be implemented and may improve diagnosis, frequency estimation, and phenotype characterisation. For example, Perez-Valencia et al used an amplicon sequencing-based MSI assay of peripheral blood leukocytes (constitutional MSI analysis) to screen for CMMRD in children suspected of sporadic NF1 who do not have a germline *NF1* or *SPRED1* PV (following comprehensive genetic testing) and who had not developed cancer, and confirmed a CMMRD diagnosis in 3/735 (0.41%, 95% CI: 0.08-1.19%) cases (Perez-Valencia et al., 2020). This empirical estimate was very similar to the estimate of 0.39% calculated by Suerink et al and hence supported their recommendations for targeted CMMRD testing in this population (Suerink et al., 2019a). Prospective studies need to be performed to evaluate the sensitivity, specificity, PPV and NPV of the guidelines proposed by Suerink et al for CMMRD in this population. CMMRD patients identified at a young age without cancer but due to their NF1-like phenotype may also provide an unbiased patient cohort for the evaluation of the natural history and the cancer risks of CMMRD.

The established CMMRD phenotype encompasses a broad tumour spectrum and age of onset. However, characterisation of CMMRD cancer risk and its modifiers is limited by the rarity of the syndrome. Previous studies have hinted at genotype-risk correlations. In 2014, Wimmer et al. reported on the cancer diagnoses of 146 CMMRD patients, and found that constitutional *PMS2* deficiency was associated with a higher incidence of brain tumours, lower incidence of haematological malignancies, and later age of onset relative to constitutional *MLH1* or *MSH2* deficiency (Wimmer et al., 2014). This is similar to the reduced penetrance of heterozygous *PMS2* variants in Lynch syndrome (Moller et al., 2017). Hypomorphic MMR variants have also been linked to less severe CMMRD phenotypes. For example, individuals homozygous for the "leaky" splice variant *PMS2* c.2002A>G p.(Ile668\*) have an attenuated CMMRD phenotype with an average age of first cancer diagnosis falling between that of CMMRD and Lynch syndrome caused by alternative *PMS2* PVs, and with a predominance of CRC diagnoses over other tumour types (Li et al., 2015). Additional,

attenuated forms of CMMRD are likely to be linked to other, specific MMR gene variants and CMMRD cancer risk is likely to be, in part, determined at the nucleotide-level of the causative germline PV. Further exploration of MMR genotype-phenotype correlations in CMMRD, including characterisation of hypomorphic variants (Gallon et al., 2024; Li et al., 2015) could facilitate better risk stratification and provide general insight into MMR function.

Although cancer surveillance in CMMRD has been shown to improve survival (Durno et al., 2021; Ghorbanoghli et al., 2023) further assessment of its efficacy and impact, as well as novel forms, need further assessment. Here, WBMRI is recommended at least once for CMMRD patients for the detection of low grade/pre-malignant tumours but the benefits of this and its use in regular surveillance are uncertain. Therefore, evaluation of the clinical utility of WBMRI in prospective studies is needed. Novel surveillance technologies for specific tumour types may also become available. For example, there is currently no recommended surveillance for urinary tract tumours in CMMRD. However, recent advancements in the context of urinary tract cancer screening in the general population or Lynch syndrome may be applicable to CMMRD, in particular the sequencing of urinary cell free DNA to detect tumour-specific MSI, somatic variants, and methylation profiles (Dudley et al., 2019; Phelps et al., 2022; Ward et al., 2023; Xiao et al., 2022). Recent data suggest surveillance for haematological malignancies is ineffective and novel approaches are needed given the exceptional risk in CMMRD. The improved overall survival of CMMRD patients due to surveillance and therapeutic advancements would mean that more patients will reach an older age. This has implications for both our understanding of the CMMRD phenotype and its management. In particular, the CMMRD cancer spectrum may change with age and surveillance protocols may need to be adapted accordingly. For example, genitourinary tract cancers may be more prevalent in adult patients (Durno et al., 2021). Surveillance recommendations include 6 monthly appointments and invasive procedures, and studies on the acceptability and psychological impact of, as well as long-term adherence to surveillance interventions are currently lacking.

CMMRD cancer risk could also be managed through prevention, though there are currently no recommendations on prevention outside of extensive colectomy for patients with colorectal polyposis or cancer, or hysterectomy for individuals who have completed childbearing. Immune-based prophylaxis may provide a novel approach to CMMRD cancer

prevention given the immunogenicity of MMR deficient tumours and the response of CMMRD tumours to ICIs (Das et al., 2022; Suerink et al., 2021b). Vaccines for MMR deficient intestinal cancer in a murine model of Lynch syndrome (MSH2 deficient intestinal tract) were found to reduce cancer incidence and mortality (Gebert et al., 2021). The murine vaccine uses frameshift peptides, the translational products of genes containing insertion-deletion variants in coding microsatellites that are recurrent in intestinal MMR deficient tumours, to stimulate an anti-tumour immune response (Hernandez-Sanchez et al., 2022). Equivalent vaccines have been developed based on frameshift peptides associated with MMR deficient cancers in humans, and have been shown to be safe in a phase I/IIa clinical trial (Kloor et al., 2020) and to induce an immune response (Kloor et al., 2020; Leoni et al., 2020). Therefore, vaccination of CMMRD patients with frameshift peptide neoantigens could be trialled in the future. Alternatively, daily acetylsalicylic acid intake approximately halved the incidence of CRC in Lynch syndrome carriers in the CAPP2 randomised control trial (Burn et al., 2020) and could be beneficial to CMMRD patients (Leenders et al., 2018). However, there is currently no strong evidence to support a reduction of cancer incidence by acetylsalicylic acid or other non-steroidal anti-inflammatory drugs in CMMRD, and further studies are needed.

Preventive effect of the use of ICIs on polyposis has been published in 2 children treated with anti-PD1 immunotherapy for medulloblastoma and gastric cancer, and showed a reduction in the GI polyps during treatment (Ozyoruk et al., 2021; Tanimura et al., 2022), while this has not been observed in other anecdotal cases. In addition, in a large series of adult patients with various cancer types, inclusion of ICI in the treatment of a first primary cancer was shown to be associated with a reduced incidence of second primary cancers as compared to those patients treated without ICI (Heudel et al., 2021). This finding should lead to evaluation of whether ICI may also reduce the risk of multiple cancers in CMMRD patients.

The loss of MMR function in CMMRD tumours makes them resistant to certain chemotherapies, in particular thiopurines and methylating agents (Aquilina et al., 1990; Bignami et al., 2003; Gupta & Heinen, 2019; Karran et al., 2003; Mas-Ponte et al., 2022; Stojic et al., 2004), whilst sensitising them to ICIs (Das et al., 2022; Suerink et al., 2021b). CMMRD tumours, therefore, may require specific management and, in general, more substantial reports on the responses of different tumour types to different treatment regimens are needed. Mechanisms of therapy resistance require exploration. In particular, some CMMRD

tumours are refractory to the promising new drug class of ICIs. There are several hypotheses regarding ICI-resistance of MMR deficient tumours, including both innate and acquired mechanisms based on often complex cellular and chemical interactions. Examples include immunosuppression by different cell types, receptors, and cytokines, tumour metabolism, specifically lactate production, activation of specific signalling pathways such as the WNT/ $\beta$ -catenin pathway, and loss of antigen expression (Sahin et al., 2019). Research in this area is ongoing and our understanding is rapidly changing. As an example, it was initially thought that loss of  $\beta$ 2M expression, and so disruption of antigen presentation, may be a mechanism of ICI-resistance. However, it was found in one study that 4/7 MMR deficient CRCs with loss of  $\beta$ 2M had partial response to ICIs, whilst the other 3/7 had stable disease, showing that  $\beta$ 2M expression may not be needed for ICI response (Middha et al., 2019). Promising biomarkers for ICI response include TMB, with higher TMB being predictive of positive ICI response among MMR deficient metastatic CRCs (Schrock et al., 2019), and measures of intra-tumoural immune activity, such as Immunoscore<sup>®</sup> (El Sissy et al., 2021; Hijazi et al., 2023). Studies of biomarkers of ICI response and resistance in the context of different CMMRD tumours are needed.

Studies of CMMRD tumourigenesis pathways will improve our understanding of the CMMRD phenotype and allow us to optimise management. Frequent alterations of the Ras/MAPK pathway have been shown in hypermutated tumours (Campbell et al, *Cancer Discovery* 2021). The efficacy of MEK inhibitors as monotherapy or in combination with PD1 inhibitors is an ongoing question, which could be the subject of a therapeutic trial in the context of tumors associated with MMR-deficiency. Topics of interest include the progression of low grade into high-grade lesions, including gastrointestinal tumours and glioma (Durno et al., 2021), and the relationship between first and second tumours, specifically whether these represent related clones and whether secondary malignant neoplasia can be caused by previous cancer therapy (Karran et al., 2003; Kroeze et al., 2022; Lavoine et al., 2015; Ripperger & Schlegelberger, 2016). Systematic collection of neoplastic tissues will be particularly useful to answer these questions. Model systems, such as cell lines, organoids, and mice, may also be helpful.



## 12. GLOSSARY

Term	Description
5-FU	5-Fluorouracil
AACR	American Association for Cancer Research
ACMG	American College of Medical Genetics
ALL	Acute Lymphoblastic Leukaemia
C4CMMRD	Care for CMMRD
CALM	Café-Au-Lait Macule
CAPP2 trial	Cancer Prevention Programme 2 trial A trial looking at preventing bowel cancer with acetylsalicylic acid or resistant starch
CI	Confidence Interval
ClinGen InSiGHT	<a href="https://clinicalgenome.org/affiliation/50099/">https://clinicalgenome.org/affiliation/50099/</a>
CMMRD	Constitutional Mismatch Repair Deficiency
COSMIC	Catalogue Of Somatic Mutations In Cancer <a href="https://cancer.sanger.ac.uk/signatures/">https://cancer.sanger.ac.uk/signatures/</a>
CRC	Colorectal cancers
DNA	Deoxyribonucleic acid
DVA	Developmental Venous Anomaly
EBV	Epstein-Barr Virus
EICNHL	European Intergroup for childhood non-Hodgkin Lymphoma
ePAG	European Patient Advocacy Group
ERN	European Reference Network
ESGE	European Society of Gastrointestinal Endoscopy
FDA	Food and Drug Administration
FLAIR	Fluid-Attenuated Inversion Recovery
gDNA	Genomic DNA
GENTURIS	Genetic Tumour Risk Syndromes
GI	Gastrointestinal

<b>gMSI</b>	<u>Germline Microsatellite Instability</u>
<b>HR</b>	<u>Hazard ratio</u>
<b>ICI</b>	<u>Immune Checkpoint Inhibitors</u>
<b>Ig</b>	<u>Immunoglobulin</u>
<b>IHC</b>	<u>Immunohistochemistry</u>
<b>IRRDC</b>	<u>International Replication Repair Deficiency Consortium</u>
<b>LFS</b>	<u>Li-Fraumeni Syndrome</u>
<b>LGSS</b>	<u>Legius Syndrome</u>
<b>LOGIC</b>	<u>Low coverage whole genome instability characterization</u>
<b>LPV</b>	<u>Likely Pathogenic Variant</u>
<b>LS</b>	<u>Lynch Syndrome</u>
<b>Mb</b>	<u>Megabase</u>
<b><i>MLH1</i></b>	<u>Mut protein Homolog 1</u> <a href="https://www.genecards.org/cgi-bin/carddisp.pl?gene=MLH1">https://www.genecards.org/cgi-bin/carddisp.pl?gene=MLH1</a>
<b>MMR</b>	<u>Mismatch Repair</u>
<b>MMRd</b>	<u>Mismatch Repair-deficient</u>
<b>MRI</b>	<u>Magnetic Resonance Imaging</u>
<b><i>MSH2</i></b>	<u>MutS protein Homolog 2</u> <a href="https://www.genecards.org/cgi-bin/carddisp.pl?gene=MSH2">https://www.genecards.org/cgi-bin/carddisp.pl?gene=MSH2</a>
<b><i>MSH6</i></b>	<u>MutS protein Homolog 6</u> <a href="https://www.genecards.org/cgi-bin/carddisp.pl?gene=MSH6">https://www.genecards.org/cgi-bin/carddisp.pl?gene=MSH6</a>
<b>MSI</b>	<u>Microsatellite Instability</u>
<b>MS-indels</b>	<u>Microsatellite insertions/deletions</u>
<b>Mut</b>	<u>Mutation(s)</u>
<b>NF1 (syndrome)</b>	<u>Neurofibromatosis type 1</u>
<b><i>NF1</i> (gene)</b>	<u>Neurofibromin 1</u> <a href="https://www.genecards.org/cgi-bin/carddisp.pl?gene=NF1">https://www.genecards.org/cgi-bin/carddisp.pl?gene=NF1</a>
<b>NGS</b>	<u>Next Generation Sequencing</u>
<b>NHL</b>	<u>Non-Hodgkin Lymphoma</u>
<b>NPV</b>	<u>Negative Predictive Value</u>

<b>Ø</b>	Diameter
<b>OS</b>	<u>O</u> verall <u>S</u> urvival
<b>PD-1</b>	<u>P</u> rogrammed cell <u>d</u> eath protein <u>1</u> Encoded by the <i>PDCD1</i> gene: <a href="https://www.genecards.org/cgi-bin/carddisp.pl?gene=PDCD1">https://www.genecards.org/cgi-bin/carddisp.pl?gene=PDCD1</a>
<b>PMS2</b>	<u>P</u> ost <u>m</u> eiotic <u>S</u> egregation Increased 2 <a href="https://www.genecards.org/cgi-bin/carddisp.pl?gene=PMS2">https://www.genecards.org/cgi-bin/carddisp.pl?gene=PMS2</a>
<b>PMS2CL</b>	<u>P</u> MS2 <u>C</u> -terminal <u>L</u> ike Pseudogene <a href="https://www.genecards.org/cgi-bin/carddisp.pl?gene=PMS2CL">https://www.genecards.org/cgi-bin/carddisp.pl?gene=PMS2CL</a>
<b>POLD1</b>	DNA <u>P</u> olymerase <u>D</u> elta 1 <a href="https://www.genecards.org/cgi-bin/carddisp.pl?gene=POLD1">https://www.genecards.org/cgi-bin/carddisp.pl?gene=POLD1</a>
<b>POLE</b>	DNA <u>P</u> olymerase <u>E</u> psilon <a href="https://www.genecards.org/cgi-bin/carddisp.pl?gene=POLE">https://www.genecards.org/cgi-bin/carddisp.pl?gene=POLE</a>
<b>PPV</b>	<u>P</u> ositive <u>P</u> redictive <u>V</u> alue
<b>PV</b>	<u>P</u> athogenic <u>V</u> ariant
<b>Rec.</b>	<u>R</u> ecommendation
<b>RNA</b>	<u>R</u> ibonucleic acid
<b>SBS</b>	<u>S</u> ingle <u>B</u> ase <u>S</u> ubstitution
<b>SFCE</b>	<u>S</u> ociété <u>F</u> rançaise <u>C</u> ancers <u>E</u> nfant (French paediatric oncology society)
<b>sPNET</b>	<u>S</u> upratentorial <u>P</u> rimitive <u>N</u> euroectodermal <u>T</u> umours
<b>SPRED1</b>	<u>S</u> prouty <u>R</u> elated <u>E</u> VH1 <u>D</u> omain Containing 1 <a href="https://www.genecards.org/cgi-bin/carddisp.pl?gene=SPRED1">https://www.genecards.org/cgi-bin/carddisp.pl?gene=SPRED1</a>
<b>T-LBL</b>	<u>T</u> -cell <u>L</u> ymphoblastic <u>L</u> ymphoma
<b>TMB</b>	<u>T</u> umour <u>M</u> utation <u>B</u> urden
<b>VCE</b>	<u>V</u> ideo <u>C</u> apsule <u>E</u> ndoscopy
<b>VUS</b>	<u>V</u> ariant of <u>U</u> nknown <u>S</u> ignificance
<b>WBMRI</b>	<u>W</u> hole- <u>b</u> ody <u>M</u> agnetic <u>R</u> esonance <u>I</u> maging
<b>WES</b>	<u>W</u> hole <u>E</u> xome <u>S</u> equencing
<b>WGS</b>	<u>W</u> hole <u>G</u> enome <u>S</u> equencing
<b>WHO</b>	<u>W</u> orld <u>H</u> ealth <u>O</u> rganization

## 13. BIBLIOGRAPHY

- Abidi, A., Gorris, M. A. J., Brennan, E., Jongmans, M. C. J., Weijers, D. D., Kuiper, R. P., de Voer, R. M., Hoogerbrugge, N., Schreibelt, G., & de Vries, I. J. M. (2021). Challenges of Neoantigen Targeting in Lynch Syndrome and Constitutional Mismatch Repair Deficiency Syndrome. *Cancers (Basel)*, *13*(10).  
<https://doi.org/10.3390/cancers13102345>
- Aggarwal, N., Quaglia, A., McPhail, M. J. W., & Monahan, K. J. (2022). Systematic review and meta-analysis of tumour microsatellite-instability status as a predictor of response to fluorouracil-based adjuvant chemotherapy in colorectal cancer. *Int J Colorectal Dis*, *37*(1), 35-46.  
<https://doi.org/10.1007/s00384-021-04046-x>
- Ahadova, A., Gallon, R., Gebert, J., Ballhausen, A., Endris, V., Kirchner, M., Stenzinger, A., Burn, J., von Knebel Doeberitz, M., Blaker, H., & Kloor, M. (2018). Three molecular pathways model colorectal carcinogenesis in Lynch syndrome. *Int J Cancer*, *143*(1), 139-150.  
<https://doi.org/10.1002/ijc.31300>
- Alexandrov, L. B., Kim, J., Haradhvala, N. J., Huang, M. N., Tian Ng, A. W., Wu, Y., Boot, A., Covington, K. R., Gordenin, D. A., Bergstrom, E. N., Islam, S. M. A., Lopez-Bigas, N., Klimczak, L. J., McPherson, J. R., Morganella, S., Sabarinathan, R., Wheeler, D. A., Mustonen, V., Group, P. M. S. W., . . . Consortium, P. (2020). The repertoire of mutational signatures in human cancer. *Nature*, *578*(7793), 94-101.  
<https://doi.org/10.1038/s41586-020-1943-3>
- AlHarbi, M., Ali Mobark, N., AlMubarak, L., Aljelaify, R., AlSaeed, M., Almutairi, A., Alqubaishi, F., Hussain, M. E., Balbaid, A. A. O., Said Marie, A., AlSubaie, L., AlShieban, S., alTassan, N., Ramkissoon, S. H., & Abedalthagafi, M. (2018). Durable Response to Nivolumab in a Pediatric Patient with Refractory Glioblastoma and Constitutional Biallelic Mismatch Repair Deficiency. *Oncologist*, *23*(12), 1401-1406.  
<https://doi.org/10.1634/theoncologist.2018-0163>
- Alotaibi, H., Ricciardone, M. D., & Ozturk, M. (2008). Homozygosity at variant MLH1 can lead to secondary mutation in NF1, neurofibromatosis type I and early onset leukemia. *Mutat Res*, *637*(1-2), 209-214.  
<https://doi.org/10.1016/j.mrfmmm.2007.08.003>
- Amayiri, N., Tabori, U., Campbell, B., Bakry, D., Aronson, M., Durno, C., Rakopoulos, P., Malkin, D., Qaddoumi, I., Musharbash, A., Swaidan, M., Bouffet, E., Hawkins, C., Al-Hussaini, M., & Consortium, B. (2016). High frequency of mismatch repair deficiency among pediatric high grade gliomas in Jordan. *Int J Cancer*, *138*(2), 380-385.  
<https://doi.org/10.1002/ijc.29724>
- Andre, T., Shiu, K. K., Kim, T. W., Jensen, B. V., Jensen, L. H., Punt, C., Smith, D., Garcia-Carbonero, R., Benavides, M., Gibbs, P., de la Fouchardiere, C., Rivera, F., Elez, E., Bendell, J., Le, D. T., Yoshino, T., Van Cutsem, E., Yang, P., Farooqui, M. Z. H., . . . Investigators, K.-. (2020). Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. *N Engl J Med*, *383*(23), 2207-2218.  
<https://doi.org/10.1056/NEJMoa2017699>
- Andrianova, M. A., Chetan, G. K., Sibin, M. K., McKee, T., Merkler, D., Narasinga, R. K., Ribaux, P., Blouin, J. L., Makrythanasis, P., Seplyarskiy, V. B., Antonarakis, S. E., & Nikolaev, S. I. (2017). Germline PMS2 and somatic POLE exonuclease mutations cause hypermutability of the leading DNA strand in biallelic mismatch repair deficiency syndrome brain tumours. *J Pathol*, *243*(3), 331-341.  
<https://doi.org/10.1002/path.4957>
- Aquilina, G., Giammarioli, A. M., Zijno, A., Di Muccio, A., Dogliotti, E., & Bignami, M. (1990). Tolerance to O6-methylguanine and 6-thioguanine cytotoxic effects: a cross-resistant phenotype in N-methylnitrosourea-resistant Chinese hamster ovary cells. *Cancer Res*, *50*(14), 4248-4253.  
<https://www.ncbi.nlm.nih.gov/pubmed/2364383>

Aronson, M., Colas, C., Shuen, A., Hampel, H., Foulkes, W. D., Baris Feldman, H., Goldberg, Y., Muleris, M., Wolfe Schneider, K., McGee, R. B., Jaspersen, K., Rangaswami, A., Brugieres, L., & Tabori, U. (2022). Diagnostic criteria for constitutional mismatch repair deficiency (CMMRD): recommendations from the international consensus working group. *J Med Genet*, *59*(4), 318-327.  
<https://doi.org/10.1136/jmedgenet-2020-107627>

Aronson, M., Gallinger, S., Cohen, Z., Cohen, S., Dvir, R., Elhasid, R., Baris, H. N., Kariv, R., Druker, H., Chan, H., Ling, S. C., Kortan, P., Holter, S., Semotiuk, K., Malkin, D., Farah, R., Sayad, A., Heald, B., Kalady, M. F., . . . Durno, C. (2016). Gastrointestinal Findings in the Largest Series of Patients With Hereditary Biallelic Mismatch Repair Deficiency Syndrome: Report from the International Consortium. *Am J Gastroenterol*, *111*(2), 275-284.  
<https://doi.org/10.1038/ajg.2015.392>

Attarbaschi, A., Carraro, E., Ronceray, L., Andres, M., Barzilai-Birenboim, S., Bomken, S., Brugieres, L., Burkhardt, B., Ceppi, F., Chiang, A. K. S., Csoka, M., Fedorova, A., Jazbec, J., Kabickova, E., Loeffen, J., Mellgren, K., Miakova, N., Moser, O., Osumi, T., . . . the International Berlin-Frankfurt-Munster Study, G. (2021). Second malignant neoplasms after treatment of non-Hodgkin's lymphoma-a retrospective multinational study of 189 children and adolescents. *Leukemia*, *35*(2), 534-549.  
<https://doi.org/10.1038/s41375-020-0841-x>

Baig, S. M., Fatima, A., Tariq, M., Khan, T. N., Ali, Z., Faheem, M., Mahmood, H., Killela, P., Waitkus, M., He, Y., Zhao, F., Wang, S., Jiao, Y., & Yan, H. (2019). Hereditary brain tumor with a homozygous germline mutation in PMS2: pedigree analysis and prenatal screening in a family with constitutional mismatch repair deficiency (CMMRD) syndrome. *Fam Cancer*, *18*(2), 261-265.  
<https://doi.org/10.1007/s10689-018-0112-4>

Bakry, D., Aronson, M., Durno, C., Rimawi, H., Farah, R., Alharbi, Q. K., Alharbi, M., Shamvil, A., Ben-Shachar, S., Mistry, M., Constantini, S., Dvir, R., Qaddoumi, I., Gallinger, S., Lerner-Ellis, J., Pollett, A., Stephens, D., Kelies, S., Chao, E., . . . Tabori, U. (2014). Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: report from the constitutional mismatch repair deficiency consortium. *Eur J Cancer*, *50*(5), 987-996.  
<https://doi.org/10.1016/j.ejca.2013.12.005>

Ballinger, M. L., Ferris, N. J., Moodie, K., Mitchell, G., Shanley, S., James, P. A., & Thomas, D. M. (2017). Surveillance in Germline TP53 Mutation Carriers Utilizing Whole-Body Magnetic Resonance Imaging. *JAMA Oncol*, *3*(12), 1735-1736.  
<https://doi.org/10.1001/jamaoncol.2017.1355>

Balmana, J., Balaguer, F., Cervantes, A., Arnold, D., & Group, E. G. W. (2013). Familial risk-colorectal cancer: ESMO Clinical Practice Guidelines. *Ann Oncol*, *24* Suppl 6, vi73-80.  
<https://doi.org/10.1093/annonc/mdt209>

Baris, H. N., Barnes-Kedar, I., Toledano, H., Halpern, M., Hershkovitz, D., Lossos, A., Lerer, I., Peretz, T., Kariv, R., Cohen, S., Half, E. E., Magal, N., Drasinover, V., Wimmer, K., Goldberg, Y., Bercovich, D., & Levi, Z. (2016). Constitutional Mismatch Repair Deficiency in Israel: High Proportion of Founder Mutations in MMR Genes and Consanguinity. *Pediatr Blood Cancer*, *63*(3), 418-427.  
<https://doi.org/10.1002/pbc.25818>

Berrino, E., Filippi, R., Visintin, C., Peirone, S., Fenocchio, E., Farinea, G., Veglio, F., Aglietta, M., Sapino, A., Cereda, M., Visintin, R., Pasini, B., & Marchio, C. (2022). Collision of germline POLE and PMS2 variants in a young patient treated with immune checkpoint inhibitors. *NPJ Precis Oncol*, *6*(1), 15.  
<https://doi.org/10.1038/s41698-022-00258-8>

Bignami, M., Casorelli, I., & Karran, P. (2003). Mismatch repair and response to DNA-damaging antitumour therapies. *Eur J Cancer*, *39*(15), 2142-2149.  
[https://doi.org/10.1016/s0959-8049\(03\)00569-0](https://doi.org/10.1016/s0959-8049(03)00569-0)

Biller, J. A., Butros, S. R., Chan-Smutko, G., Abrams, A. N., Chung, D. C., & Hagen, C. E. (2016). CASE RECORDS of the MASSACHUSETTS GENERAL HOSPITAL. Case 6-2016. A 10-Year-Old Boy with Abdominal Cramping and Fevers. *N Engl J Med*, *374*(8), 772-781.  
<https://doi.org/10.1056/NEJMcpc1408597>

Bodo, S., Colas, C., Buhard, O., Collura, A., Tinat, J., Lavoine, N., Guilloux, A., Chalastanis, A., Lafitte, P., Coulet, F., Buisine, M. P., Ilencikova, D., Ruiz-Ponte, C., Kinzel, M., Grandjouan, S., Brems, H., Lejeune, S., Blanche, H., Wang, Q., . . . European Consortium "Care for, C. (2015). Diagnosis of Constitutional Mismatch Repair-Deficiency Syndrome Based on Microsatellite Instability and Lymphocyte Tolerance to Methylating Agents. *Gastroenterology*, *149*(4), 1017-1029 e1013.  
<https://doi.org/10.1053/j.gastro.2015.06.013>

Bouffet, E., Larouche, V., Campbell, B. B., Merico, D., de Borja, R., Aronson, M., Durno, C., Krueger, J., Cabric, V., Ramaswamy, V., Zhukova, N., Mason, G., Farah, R., Afzal, S., Yalon, M., Rechavi, G., Magimairajan, V., Walsh, M. F., Constantini, S., . . . Tabori, U. (2016). Immune Checkpoint Inhibition for Hypermutant Glioblastoma Multiforme Resulting From Germline Biallelic Mismatch Repair Deficiency. *J Clin Oncol*, *34*(19), 2206-2211.  
<https://doi.org/10.1200/JCO.2016.66.6552>

Bruekner, S. R., Pieters, W., Fish, A., Liaci, A. M., Scheffers, S., Rayner, E., Kaldenbach, D., Drost, L., Dekker, M., van Hees-Stuivenberg, S., Delzenne-Goette, E., de Konink, C., Houlleberghs, H., Dubbink, H. J., AlSaegh, A., de Wind, N., Forster, F., Te Riele, H., & Sixma, T. K. (2023). Unexpected moves: a conformational change in MutSalpha enables high-affinity DNA mismatch binding. *Nucleic Acids Res*, *51*(3), 1173-1188.  
<https://doi.org/10.1093/nar/gkado15>

Bruwer, Z., Algar, U., Vorster, A., Fieggen, K., Davidson, A., Goldberg, P., Wainwright, H., & Ramesar, R. (2014). Predictive genetic testing in children: constitutional mismatch repair deficiency cancer predisposing syndrome. *J Genet Couns*, *23*(2), 147-155.  
<https://doi.org/10.1007/s10897-013-9659-2>

Burn, J., Sheth, H., Elliott, F., Reed, L., Macrae, F., Mecklin, J. P., Moslein, G., McDonald, F. E., Bertario, L., Evans, D. G., Gerdes, A. M., Ho, J. W. C., Lindblom, A., Morrison, P. J., Rashbass, J., Ramesar, R., Seppala, T., Thomas, H. J. W., Pylvanainen, K., . . . Investigators, C. (2020). 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*, *395*(10240), 1855-1863.  
[https://doi.org/10.1016/S0140-6736\(20\)30366-4](https://doi.org/10.1016/S0140-6736(20)30366-4)

Cancer Genome Atlas, N. (2012). Comprehensive molecular characterization of human colon and rectal cancer. *Nature*, *487*(7407), 330-337.  
<https://doi.org/10.1038/nature11252>

Carethers, J. M., Chauhan, D. P., Fink, D., Nebel, S., Bresalier, R. S., Howell, S. B., & Boland, C. R. (1999). Mismatch repair proficiency and in vitro response to 5-fluorouracil. *Gastroenterology*, *117*(1), 123-131.  
[https://doi.org/10.1016/S0016-5085\(99\)70558-5](https://doi.org/10.1016/S0016-5085(99)70558-5)

Carrato, C., Sanz, C., Munoz-Marmol, A. M., Blanco, I., Pineda, M., Del Valle, J., Damaso, E., Esteller, M., & Musulen, E. (2021). The Challenge of Diagnosing Constitutional Mismatch Repair Deficiency Syndrome in Brain Malignancies from Young Individuals. *Int J Mol Sci*, *22*(9).  
<https://doi.org/10.3390/ijms22094629>

Cercek, A., Lumish, M., Sinopoli, J., Weiss, J., Shia, J., Lamendola-Essel, M., El Dika, I. H., Segal, N., Shcherba, M., Sugarman, R., Stadler, Z., Yaeger, R., Smith, J. J., Rousseau, B., Argiles, G., Patel, M., Desai, A., Saltz, L. B., Widmar, M., . . . Diaz, L. A., Jr. (2022). PD-1 Blockade in Mismatch Repair-Deficient, Locally Advanced Rectal Cancer. *N Engl J Med*, *386*(25), 2363-2376.  
<https://doi.org/10.1056/NEJMOa2201445>

Chung, J., Maruvka, Y. E., Sudhaman, S., Kelly, J., Haradhvala, N. J., Bianchi, V., Edwards, M., Forster, V. J., Nunes, N. M., Galati, M. A., Komosa, M., Deshmukh, S., Cabric, V., Davidson, S., Zatzman, M., Light, N., Hayes, R., Brunga, L., Anderson, N. D., . . . Tabori, U. (2021). DNA Polymerase and Mismatch Repair Exert Distinct Microsatellite Instability Signatures in Normal and Malignant Human Cells. *Cancer Discov*, 11(5), 1176-1191.

<https://doi.org/10.1158/2159-8290.CD-20-0790>

Chung, J., Negm, L., Bianchi, V., Stengs, L., Das, A., Liu, Z. A., Sudhaman, S., Aronson, M., Brunga, L., Edwards, M., Forster, V., Komosa, M., Davidson, S., Lees, J., Tomboc, P., Samuel, D., Farah, R., Bendel, A., Knipstein, J., . . . International Replication Repair Deficiency, C. (2023). Genomic Microsatellite Signatures Identify Germline Mismatch Repair Deficiency and Risk of Cancer Onset. *J Clin Oncol*, 41(4), 766-777.

<https://doi.org/10.1200/JCO.21.02873>

Cloughesy, T. F., Mochizuki, A. Y., Orpilla, J. R., Hugo, W., Lee, A. H., Davidson, T. B., Wang, A. C., Ellingson, B. M., Rytlewski, J. A., Sanders, C. M., Kawaguchi, E. S., Du, L., Li, G., Yong, W. H., Gaffey, S. C., Cohen, A. L., Mellinshoff, I. K., Lee, E. Q., Reardon, D. A., . . . Prins, R. M. (2019). Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. *Nat Med*, 25(3), 477-486.

<https://doi.org/10.1038/s41591-018-0337-7>

Das, A., Sudhaman, S., Morgenstern, D., Coblentz, A., Chung, J., Stone, S. C., Alsafwani, N., Liu, Z. A., Karsaneh, O. A. A., Soleimani, S., Ladany, H., Chen, D., Zatzman, M., Cabric, V., Nobre, L., Bianchi, V., Edwards, M., Sambira Nahum, L. C., Ercan, A. B., . . . Tabori, U. (2022). Genomic predictors of response to PD-1 inhibition in children with germline DNA replication repair deficiency. *Nat Med*, 28(1), 125-135.

<https://doi.org/10.1038/s41591-021-01581-6>

de Voer, R. M., Diets, I. J., van der Post, R. S., Weren, R. D. A., Kamping, E. J., de Bitter, T. J. J., Elze, L., Verhoeven, R. H. A., Vink-Borger, E., Eijkelenboom, A., Mensenkamp, A., Nagtegaal, I. D., Jongmans, M. C. J., & Ligtenberg, M. J. L. (2021). Clinical, Pathology, Genetic, and Molecular Features of Colorectal Tumors in Adolescents and Adults 25 Years or Younger. *Clin Gastroenterol Hepatol*, 19(8), 1642-1651 e1648.

<https://doi.org/10.1016/j.cgh.2020.06.034>

Dominguez-Valentin, M., Sampson, J. R., Seppala, T. T., Ten Broeke, S. W., Plazzer, J. P., Nakken, S., Engel, C., Aretz, S., Jenkins, M. A., Sunde, L., Bernstein, I., Capella, G., Balaguer, F., Thomas, H., Evans, D. G., Burn, J., Greenblatt, M., Hovig, E., de Vos Tot Nederveen Cappel, W. H., . . . Moller, P. (2020). Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. *Genet Med*, 22(1), 15-25.

<https://doi.org/10.1038/s41436-019-0596-9>

Dudley, J. C., Schroers-Martin, J., Lazzareschi, D. V., Shi, W. Y., Chen, S. B., Esfahani, M. S., Trivedi, D., Chabon, J. J., Chaudhuri, A. A., Stehr, H., Liu, C. L., Lim, H., Costa, H. A., Nabet, B. Y., Sin, M. L. Y., Liao, J. C., Alizadeh, A. A., & Diehn, M. (2019). Detection and Surveillance of Bladder Cancer Using Urine Tumor DNA. *Cancer Discov*, 9(4), 500-509.

<https://doi.org/10.1158/2159-8290.CD-18-0825>

Durno, C., Boland, C. R., Cohen, S., Dominitz, J. A., Giardiello, F. M., Johnson, D. A., Kaltenbach, T., Levin, T. R., Lieberman, D., Robertson, D. J., & Rex, D. K. (2017a). Recommendations on surveillance and management of biallelic mismatch repair deficiency (BMMRD) syndrome: a consensus statement by the US Multi-Society Task Force on Colorectal Cancer. *Gastrointest Endosc*, 85(5), 873-882.

<https://doi.org/10.1016/j.gie.2017.03.015>

Durno, C., Boland, C. R., Cohen, S., Dominitz, J. A., Giardiello, F. M., Johnson, D. A., Kaltenbach, T., Levin, T. R., Lieberman, D., Robertson, D. J., & Rex, D. K. (2017b). Recommendations on Surveillance and Management of Biallelic Mismatch Repair Deficiency (BMMRD) Syndrome: A Consensus

Statement by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology*, 152(6), 1605-1614.

<https://doi.org/10.1053/j.gastro.2017.02.011>

Durno, C., Ercan, A. B., Bianchi, V., Edwards, M., Aronson, M., Galati, M., Atenafu, E. G., Abebe-Campino, G., Al-Battashi, A., Alharbi, M., Azad, V. F., Baris, H. N., Basel, D., Bedgood, R., Bendel, A., Ben-Shachar, S., Blumenthal, D. T., Blundell, M., Bornhorst, M., . . . Tabori, U. (2021). Survival Benefit for Individuals With Constitutional Mismatch Repair Deficiency Undergoing Surveillance. *J Clin Oncol*, 39(25), 2779-2790.

<https://doi.org/10.1200/JCO.20.02636>

Durno, C. A., Sherman, P. M., Aronson, M., Malkin, D., Hawkins, C., Bakry, D., Bouffet, E., Gallinger, S., Pollett, A., Campbell, B., Tabori, U., & International, B. C. (2015). Phenotypic and genotypic characterisation of biallelic mismatch repair deficiency (BMMR-D) syndrome. *Eur J Cancer*, 51(8), 977-983.

<https://doi.org/10.1016/j.ejca.2015.02.008>

El Sissy, C., Kirilovsky, A., Zeitoun, G., Marliot, F., Haicheur, N., Lagorce-Pages, C., Galon, J., & Pages, F. (2021). Therapeutic Implications of the Immunoscore in Patients with Colorectal Cancer. *Cancers (Basel)*, 13(6).

<https://doi.org/10.3390/cancers13061281>

Elhasid, R., Dvir, R., Rosenfeld Keidar, H., Ben Shachar, S., Bitan, M., Solar, I., Durno, C., Aronson, M., Malkin, D., Hawkins, C., Bouffet, E., Tabori, U., & Consortium, B. (2015). Management of Acute Myeloblastic Leukemia in a Child With Biallelic Mismatch Repair Deficiency. *J Pediatr Hematol Oncol*, 37(8), e490-493.

<https://doi.org/10.1097/MPH.0000000000000415>

Ercan, A. B., Aronson, M., Fernandez, N. R., Chang, Y., Levine, A., Liu, Z. A., Negm, L., Edwards, M., Bianchi, V., Stengs, L., Chung, J., Al-Battashi, A., Reschke, A., Lion, A., Ahmad, A., Lassaletta, A., Reddy, A. T., Al-Darraj, A. F., Shah, A. C., . . . Tabori, U. (2024). Clinical and biological landscape of constitutional mismatch-repair deficiency syndrome: an International Replication Repair Deficiency Consortium cohort study. *Lancet Oncol*, 25(5), 668-682.

[https://doi.org/10.1016/S1470-2045\(24\)00026-3](https://doi.org/10.1016/S1470-2045(24)00026-3)

Etzler, J., Peyrl, A., Zatkova, A., Schildhaus, H. U., Ficek, A., Merkelbach-Bruse, S., Kratz, C. P., Attarbaschi, A., Hainfellner, J. A., Yao, S., Messiaen, L., Slavic, I., & Wimmer, K. (2008). RNA-based mutation analysis identifies an unusual MSH6 splicing defect and circumvents PMS2 pseudogene interference. *Hum Mutat*, 29(2), 299-305.

<https://doi.org/10.1002/humu.20657>

Frebourg, T., Bajalica Lagercrantz, S., Oliveira, C., Magenheimer, R., Evans, D. G., & European Reference Network, G. (2020). Guidelines for the Li-Fraumeni and heritable TP53-related cancer syndromes. *Eur J Hum Genet*, 28(10), 1379-1386.

<https://doi.org/10.1038/s41431-020-0638-4>

Gallon, R., Brekelmans, C., Martin, M., Bours, V., Schamschula, E., Amberger, A., Muleris, M., Colas, C., Dekervel, J., De Hertogh, G., Coupier, J., Colleye, O., Sepulchre, E., Burn, J., Brems, H., Legius, E., & Wimmer, K. (2024). Constitutional mismatch repair deficiency mimicking Lynch syndrome is associated with hypomorphic mismatch repair gene variants. *NPJ Precis Oncol*, 8(1), 119.

<https://doi.org/10.1038/s41698-024-00603-z>

Gallon, R., Muhlegger, B., Wenzel, S. S., Sheth, H., Hayes, C., Aretz, S., Dahan, K., Foulkes, W., Kratz, C. P., Ripperger, T., Azizi, A. A., Baris Feldman, H., Chong, A. L., Demirsoy, U., Florkin, B., Imschweiler, T., Januszkiewicz-Lewandowska, D., Lobitz, S., Nathrath, M., . . . Wimmer, K. (2019). A sensitive and scalable microsatellite instability assay to diagnose constitutional mismatch repair deficiency by sequencing of peripheral blood leukocytes. *Hum Mutat*, 40(5), 649-655.

<https://doi.org/10.1002/humu.23721>



Gallon, R., Phelps, R., Hayes, C., Brugieres, L., Guerrini-Rousseau, L., Colas, C., Muleris, M., Ryan, N. A. J., Evans, D. G., Grice, H., Jessop, E., Kunzemann-Martinez, A., Marshall, L., Schamschula, E., Oberhuber, K., Azizi, A. A., Baris Feldman, H., Beilken, A., Brauer, N., . . . Santibanez-Koref, M. (2023). Constitutional Microsatellite Instability, Genotype, and Phenotype Correlations in Constitutional Mismatch Repair Deficiency. *Gastroenterology*, *164*(4), 579-592 e578.  
<https://doi.org/10.1053/j.gastro.2022.12.017>

Gan, T., Wang, Y., Xie, M., Wang, Q., Zhao, S., Wang, P., Shi, Q., Qian, X., Miao, F., Shen, Z., & Nie, E. (2022). MEX3A Impairs DNA Mismatch Repair Signaling and Mediates Acquired Temozolomide Resistance in Glioblastoma. *Cancer Res*, *82*(22), 4234-4246.  
<https://doi.org/10.1158/0008-5472.CAN-22-2036>

Ganster, C., Wernstedt, A., Kehrer-Sawatzki, H., Messiaen, L., Schmidt, K., Rahner, N., Heinimann, K., Fonatsch, C., Zschocke, J., & Wimmer, K. (2010). Functional PMS2 hybrid alleles containing a pseudogene-specific missense variant trace back to a single ancient intrachromosomal recombination event. *Hum Mutat*, *31*(5), 552-560.  
<https://doi.org/10.1002/humu.21223>

Gebert, J., Gelincik, O., Oezcan-Wahlbrink, M., Marshall, J. D., Hernandez-Sanchez, A., Urban, K., Long, M., Cortes, E., Tosti, E., Katzenmaier, E. M., Song, Y., Elsaadi, A., Deng, N., Vilar, E., Fuchs, V., Nelius, N., Yuan, Y. P., Ahadova, A., Sei, S., . . . Kloor, M. (2021). Recurrent Frameshift Neoantigen Vaccine Elicits Protective Immunity With Reduced Tumor Burden and Improved Overall Survival in a Lynch Syndrome Mouse Model. *Gastroenterology*, *161*(4), 1288-1302 e1213.  
<https://doi.org/10.1053/j.gastro.2021.06.073>

Ghorbanoghli, Z., van Kouwen, M., Versluys, B., Bonnet, D., Devalck, C., Tinat, J., Januszkiewicz-Lewandowska, D., Costas, C. C., Cottureau, E., Hardwick, J. C. H., Wimmer, K., Brugieres, L., Colas, C., & Vasen, H. F. A. (2023). High yield of surveillance in patients diagnosed with constitutional mismatch repair deficiency. *J Med Genet*, *60*(7), 679-684.  
<https://doi.org/10.1136/jmg-2022-108829>

Gokce, E., Acu, B., Beyhan, M., Celikyay, F., & Celikyay, R. (2014). Magnetic resonance imaging findings of developmental venous anomalies. *Clin Neuroradiol*, *24*(2), 135-143.  
<https://doi.org/10.1007/s00062-013-0235-9>

Goldberg, Y., Barnes-Kedar, I., Lerer, I., Halpern, N., Plessner, M., Hubert, A., Kadouri, L., Goldshmidt, H., Solar, I., Strul, H., Rosner, G., Baris, H. N., Peretz, T., Levi, Z., & Kariv, R. (2015). Genetic features of Lynch syndrome in the Israeli population. *Clin Genet*, *87*(6), 549-553.  
<https://doi.org/10.1111/cge.12530>

Gonzalez-Acosta, M., Marin, F., Puliafito, B., Bonifaci, N., Fernandez, A., Navarro, M., Salvador, H., Balaguer, F., Iglesias, S., Velasco, A., Grau Garces, E., Moreno, V., Gonzalez-Granado, L. I., Guerra-Garcia, P., Ayala, R., Florquin, B., Kratz, C., Ripperger, T., Rosenbaum, T., . . . Pineda, M. (2020). High-sensitivity microsatellite instability assessment for the detection of mismatch repair defects in normal tissue of biallelic germline mismatch repair mutation carriers. *J Med Genet*, *57*(4), 269-273.  
<https://doi.org/10.1136/jmedgenet-2019-106272>

Goodenberger, M. L., Thomas, B. C., Riegert-Johnson, D., Boland, C. R., Plon, S. E., Clendenning, M., Win, A. K., Senter, L., Lipkin, S. M., Stadler, Z. K., Macrae, F. A., Lynch, H. T., Weitzel, J. N., de la Chapelle, A., Syngal, S., Lynch, P., Parry, S., Jenkins, M. A., Gallinger, S., . . . Lindor, N. M. (2016). PMS2 monoallelic mutation carriers: the known unknown. *Genet Med*, *18*(1), 13-19.  
<https://doi.org/10.1038/gim.2015.27>

Gröbner, S. N., Worst, B. C., Weischenfeldt, J., Buchhalter, I., Kleinheinz, K., Rudneva, V. A., Johann, P. D., Balasubramanian, G. P., Segura-Wang, M., Brabetz, S., Bender, S., Hutter, B., Sturm, D., Pfaff, E., Hubschmann, D., Zipprich, G., Heinold, M., Eils, J., Lawerenz, C., . . . Pfister, S. M. (2018). The landscape of genomic alterations across childhood cancers. *Nature*, *555*(7696), 321-327.  
<https://doi.org/10.1038/nature25480>

Guerrini-Rousseau, L., Pasmant, E., Muleris, M., Abbou, S., Adam-De-Baumais, T., Brugieres, L., Cabaret, O., Colas, C., Cotteret, S., Decq, P., Dufour, C., Guillerme, E., Rouleau, E., Varlet, P., Zili, S., Vidaud, D., & Grill, J. (2024). Neurofibromatosis type 1 mosaicism in patients with constitutional mismatch repair deficiency. *J Med Genet*, *61*(2), 158-162.  
<https://doi.org/10.1136/jmg-2023-109235>

Guerrini-Rousseau, L., Varlet, P., Colas, C., Andreiuolo, F., Bourdeaut, F., Dahan, K., Devalck, C., Faure-Conter, C., Genuardi, M., Goldberg, Y., Kuhlen, M., Moalla, S., Opocher, E., Perez-Alonso, V., Sehested, A., Slavic, I., Unger, S., Wimmer, K., Grill, J., & Brugieres, L. (2019). Constitutional mismatch repair deficiency-associated brain tumors: report from the European C4CMMRD consortium. *Neurooncol Adv*, *1*(1), vdz033.  
<https://doi.org/10.1093/noajnl/vdz033>

Gupta, D., & Heinen, C. D. (2019). The mismatch repair-dependent DNA damage response: Mechanisms and implications. *DNA Repair (Amst)*, *78*, 60-69.  
<https://doi.org/10.1016/j.dnarep.2019.03.009>

Hampel, H., Stephens, J. A., Pukkala, E., Sankila, R., Aaltonen, L. A., Mecklin, J. P., & de la Chapelle, A. (2005). Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. *Gastroenterology*, *129*(2), 415-421.  
<https://doi.org/10.1016/j.gastro.2005.05.011>

Har-Noy, O., Yung, D. E., Koulaouzidis, A., Eliakim, R., Kopylov, U., Avidan, B., & Katz, L. H. (2019). Chromoendoscopy or white light endoscopy for neoplasia detection in Lynch syndrome, a meta-analysis. *Dig Liver Dis*, *51*(11), 1515-1521.  
<https://doi.org/10.1016/j.dld.2019.07.018>

Haraldsdottir, S., Rafnar, T., Frankel, W. L., Einarsdottir, S., Sigurdsson, A., Hampel, H., Snaebjornsson, P., Masson, G., Weng, D., Arngrimsson, R., Kehr, B., Yilmaz, A., Haraldsson, S., Sulem, P., Stefansson, T., Shields, P. G., Sigurdsson, F., Bekaii-Saab, T., Moller, P. H., . . . Stefansson, K. (2017). Comprehensive population-wide analysis of Lynch syndrome in Iceland reveals founder mutations in MSH6 and PMS2. *Nat Commun*, *8*, 14755.  
<https://doi.org/10.1038/ncomms14755>

Hayward, B. E., De Vos, M., Valleley, E. M., Charlton, R. S., Taylor, G. R., Sheridan, E., & Bonthron, D. T. (2007). Extensive gene conversion at the PMS2 DNA mismatch repair locus. *Hum Mutat*, *28*(5), 424-430.  
<https://doi.org/10.1002/humu.20457>

Herkert, J. C., Niessen, R. C., Olderode-Berends, M. J., Veenstra-Knol, H. E., Vos, Y. J., van der Klift, H. M., Scheenstra, R., Tops, C. M., Karrenbeld, A., Peters, F. T., Hofstra, R. M., Kleibeuker, J. H., & Sijmons, R. H. (2011). Paediatric intestinal cancer and polyposis due to bi-allelic PMS2 mutations: case series, review and follow-up guidelines. *Eur J Cancer*, *47*(7), 965-982.  
<https://doi.org/10.1016/j.ejca.2011.01.013>

Hernandez-Sanchez, A., Grossman, M., Yeung, K., Sei, S. S., Lipkin, S., & Kloor, M. (2022). Vaccines for immunoprevention of DNA mismatch repair deficient cancers. *J Immunother Cancer*, *10*(6).  
<https://doi.org/10.1136/jitc-2021-004416>

Heudel, P., Chabaud, S., Perol, D., Flechon, A., Fayette, J., Combemale, P., Tredan, O., Desseigne, F., de la Fouchardiere, C., Boyle, H., Perol, M., Bachelot, T., Cassier, P., Avrillon, V., Terret, C., Michallet, A. S., Neidhardt-Berard, E. M., Nicolas-Virelizier, E., Dufresne, A., . . . Blay, J. Y. (2021). Immune checkpoint inhibitor treatment of a first cancer is associated with a decreased incidence of second primary cancer. *ESMO Open*, *6*(1), 100044.  
<https://doi.org/10.1016/j.esmoop.2020.100044>

Hijazi, A., Antoniotti, C., Cremolini, C., & Galon, J. (2023). Light on life: immunoscore immune-checkpoint, a predictor of immunotherapy response. *Oncoimmunology*, *12*(1), 2243169.  
<https://doi.org/10.1080/2162402X.2023.2243169>

- Hodel, K. P., Sun, M. J. S., Ungerleider, N., Park, V. S., Williams, L. G., Bauer, D. L., Immethun, V. E., Wang, J., Suo, Z., Lu, H., McLachlan, J. B., & Pursell, Z. F. (2020). POLE Mutation Spectra Are Shaped by the Mutant Allele Identity, Its Abundance, and Mismatch Repair Status. *Mol Cell*, *78*(6), 1166-1177 e1166.  
<https://doi.org/10.1016/j.molcel.2020.05.012>
- Huneburg, R., Heling, D., Kaczmarek, D. J., van Heteren, P., Olthaus, M., Fimmers, R., Berger, M., Coch, C., Lau, J. F., Kristiansen, G., Weismuller, T. J., Spier, I., Aretz, S., Strassburg, C. P., & Nattermann, J. (2020). Dye chromoendoscopy leads to a higher adenoma detection in the duodenum and stomach in patients with familial adenomatous polyposis. *Endosc Int Open*, *8*(10), E1308-E1314.  
<https://doi.org/10.1055/a-1220-6699>
- Ingham, D., Diggle, C. P., Berry, I., Bristow, C. A., Hayward, B. E., Rahman, N., Markham, A. F., Sheridan, E. G., Bonthron, D. T., & Carr, I. M. (2013). Simple detection of germline microsatellite instability for diagnosis of constitutional mismatch repair cancer syndrome. *Hum Mutat*, *34*(6), 847-852.  
<https://doi.org/10.1002/humu.22311>
- Jin, Z., & Sinicrope, F. A. (2022). Mismatch Repair-Deficient Colorectal Cancer: Building on Checkpoint Blockade. *J Clin Oncol*, *40*(24), 2735-2750.  
<https://doi.org/10.1200/JCO.21.02691>
- Jiricny, J. (2006). The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol*, *7*(5), 335-346.  
<https://doi.org/10.1038/nrm1907>
- Johannesma, P. C., van der Klift, H. M., van Grieken, N. C., Troost, D., Te Riele, H., Jacobs, M. A., Postma, T. J., Heideman, D. A., Tops, C. M., Wijnen, J. T., & Menko, F. H. (2011). Childhood brain tumours due to germline bi-allelic mismatch repair gene mutations. *Clin Genet*, *80*(3), 243-255.  
<https://doi.org/10.1111/j.1399-0004.2011.01635.x>
- Karran, P., Offman, J., & Bignami, M. (2003). Human mismatch repair, drug-induced DNA damage, and secondary cancer. *Biochimie*, *85*(11), 1149-1160.  
<https://doi.org/10.1016/j.biochi.2003.10.007>
- Kebudi, R., Amayiri, N., Abedalthagafi, M., Rana, A. N., Kirmani, S., Musthaq, N., Lamki, Z. A., Houdzi, J. E., Yazici, H., El-Naggar, S., Edwards, M., Bianchi, V. J., Durno, C., Tabori, U., Bouffet, E., & International, R. R. D. C. o. L.-R. S. P. (2020). Position paper: Challenges and specific strategies for constitutional mismatch repair deficiency syndrome in low-resource settings. *Pediatr Blood Cancer*, *67*(8), e28309.  
<https://doi.org/10.1002/pbc.28309>
- Kerpel, A., Yalon, M., Soudack, M., Chiang, J., Gajjar, A., Nichols, K. E., Patay, Z., Shrot, S., & Hoffmann, C. (2020). Neuroimaging Findings in Children with Constitutional Mismatch Repair Deficiency Syndrome. *AJNR Am J Neuroradiol*, *41*(5), 904-910.  
<https://doi.org/10.3174/ajnr.A6512>
- Khdair-Ahmad, O., Al Husaini, M., Ghunaimat, S., Ismael, T., Amayiri, N., Halalsheh, H., Jaara, M., & Sultan, I. (2021). Constitutional Mismatch Repair Deficiency in children with colorectal carcinoma: A Jordanian center experience. *Pediatric Hematology Oncology Journal*, *6*(1), 18-21.  
<https://doi.org/https://doi.org/10.1016/j.phoj.2020.10.004>
- Kloor, M., Reuschenbach, M., Pauligk, C., Karbach, J., Rafiyan, M. R., Al-Batran, S. E., Tariverdian, M., Jager, E., & von Knebel Doeberitz, M. (2020). A Frameshift Peptide Neoantigen-Based Vaccine for Mismatch Repair-Deficient Cancers: A Phase I/IIa Clinical Trial. *Clin Cancer Res*, *26*(17), 4503-4510.  
<https://doi.org/10.1158/1078-0432.CCR-19-3517>
- Kloor, M., & von Knebel Doeberitz, M. (2016). The Immune Biology of Microsatellite-Unstable Cancer. *Trends Cancer*, *2*(3), 121-133.  
<https://doi.org/10.1016/j.trecan.2016.02.004>

Kratz, C. P., Holter, S., Etzler, J., Lauten, M., Pollett, A., Niemeyer, C. M., Gallinger, S., & Wimmer, K. (2009). Rhabdomyosarcoma in patients with constitutional mismatch-repair-deficiency syndrome. *J Med Genet*, *46*(6), 418-420.  
<https://doi.org/10.1136/jmg.2008.064212>

Kratz, C. P., Smirnov, D., Autry, R., Jager, N., Waszak, S. M., Grosshennig, A., Berutti, R., Wendorff, M., Hainaut, P., Pfister, S. M., Prokisch, H., Ripperger, T., & Malkin, D. (2022). Heterozygous BRCA1 and BRCA2 and Mismatch Repair Gene Pathogenic Variants in Children and Adolescents With Cancer. *J Natl Cancer Inst*, *114*(11), 1523-1532.  
<https://doi.org/10.1093/jnci/djac151>

Kroeze, E., Weijers, D. D., Hagleitner, M. M., de Groot-Kruseman, H. A., Jongmans, M. C. J., Kuiper, R. P., Pieters, R., Meijerink, J. P. P., & Loeffen, J. L. C. (2022). High Prevalence of Constitutional Mismatch Repair Deficiency in a Pediatric T-cell Lymphoblastic Lymphoma Cohort. *Hemasphere*, *6*(1), e668.  
<https://doi.org/10.1097/HS9.000000000000668>

Latham, A., Srinivasan, P., Kemel, Y., Shia, J., Bandlamudi, C., Mandelker, D., Middha, S., Hechtman, J., Zehir, A., Dubard-Gault, M., Tran, C., Stewart, C., Sheehan, M., Penson, A., DeLair, D., Yaeger, R., Vijai, J., Mukherjee, S., Galle, J., . . . Stadler, Z. K. (2019). Microsatellite Instability Is Associated With the Presence of Lynch Syndrome Pan-Cancer. *J Clin Oncol*, *37*(4), 286-295.  
<https://doi.org/10.1200/JCO.18.00283>

Lavoine, N., Colas, C., Muleris, M., Bodo, S., Duval, A., Entz-Werle, N., Coulet, F., Cabaret, O., Andreiuolo, F., Charpy, C., Sebille, G., Wang, Q., Lejeune, S., Buisine, M. P., Leroux, D., Couillault, G., Leverger, G., Fricker, J. P., Guimbaud, R., . . . Brugieres, L. (2015). Constitutional mismatch repair deficiency syndrome: clinical description in a French cohort. *J Med Genet*, *52*(11), 770-778.  
<https://doi.org/10.1136/jmedgenet-2015-103299>

Le, D. T., Durham, J. N., Smith, K. N., Wang, H., Bartlett, B. R., Aulakh, L. K., Lu, S., Kemberling, H., Wilt, C., Lubner, B. S., Wong, F., Azad, N. S., Rucki, A. A., Laheru, D., Donehower, R., Zaheer, A., Fisher, G. A., Crocenzi, T. S., Lee, J. J., . . . Diaz, L. A., Jr. (2017). Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*, *357*(6349), 409-413.  
<https://doi.org/10.1126/science.aan6733>

Le, D. T., Uram, J. N., Wang, H., Bartlett, B. R., Kemberling, H., Eyring, A. D., Skora, A. D., Lubner, B. S., Azad, N. S., Laheru, D., Biedrzycki, B., Donehower, R. C., Zaheer, A., Fisher, G. A., Crocenzi, T. S., Lee, J. J., Duffy, S. M., Goldberg, R. M., de la Chapelle, A., . . . Diaz, L. A., Jr. (2015). PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med*, *372*(26), 2509-2520.  
<https://doi.org/10.1056/NEJMoa1500596>

Leenders, E., Westdorp, H., Bruggemann, R. J., Loeffen, J., Kratz, C., Burn, J., Hoogerbrugge, N., & Jongmans, M. C. J. (2018). Cancer prevention by aspirin in children with Constitutional Mismatch Repair Deficiency (CMMRD). *Eur J Hum Genet*, *26*(10), 1417-1423.  
<https://doi.org/10.1038/s41431-018-0197-0>

Legius, E., Messiaen, L., Wolkenstein, P., Pancza, P., Avery, R. A., Berman, Y., Blakeley, J., Babovic-Vuksanovic, D., Cunha, K. S., Ferner, R., Fisher, M. J., Friedman, J. M., Gutmann, D. H., Kehrer-Sawatzki, H., Korf, B. R., Mautner, V. F., Peltonen, S., Rauen, K. A., Riccardi, V., . . . Plotkin, S. R. (2021). Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. *Genet Med*, *23*(8), 1506-1513.  
<https://doi.org/10.1038/s41436-021-01170-5>

Leoni, G., D'Alise, A. M., Cotugno, G., Langone, F., Garzia, I., De Lucia, M., Fichera, I., Vitale, R., Bignone, V., Tucci, F. G., Mori, F., Leuzzi, A., Di Matteo, E., Troise, F., Abbate, A., Merone, R., Ruzza, V., Diodoro, M. G., Yadav, M., . . . Scarselli, E. (2020). A Genetic Vaccine Encoding Shared Cancer Neoantigens to Treat Tumors with Microsatellite Instability. *Cancer Res*, *80*(18), 3972-3982.  
<https://doi.org/10.1158/0008-5472.CAN-20-1072>

- Levi, Z., Kariv, R., Barnes-Kedar, I., Goldberg, Y., Half, E., Morgentern, S., Eli, B., Baris, H. N., Vilkin, A., Belfer, R. G., Niv, Y., Elhasid, R., Dvir, R., Abu-Freha, N., & Cohen, S. (2015). The gastrointestinal manifestation of constitutional mismatch repair deficiency syndrome: from a single adenoma to polyposis-like phenotype and early onset cancer. *Clin Genet*, *88*(5), 474-478.  
<https://doi.org/10.1111/cge.12518>
- Li, G. M. (2008). Mechanisms and functions of DNA mismatch repair. *Cell Res*, *18*(1), 85-98.  
<https://doi.org/10.1038/cr.2007.115>
- Li, L., Hamel, N., Baker, K., McGuffin, M. J., Couillard, M., Gologan, A., Marcus, V. A., Chodirker, B., Chudley, A., Stefanovici, C., Durandy, A., Hegele, R. A., Feng, B. J., Goldgar, D. E., Zhu, J., De Rosa, M., Gruber, S. B., Wimmer, K., Young, B., . . . Foulkes, W. D. (2015). A homozygous PMS2 founder mutation with an attenuated constitutional mismatch repair deficiency phenotype. *J Med Genet*, *52*(5), 348-352.  
<https://doi.org/10.1136/jmedgenet-2014-102934>
- Lindsay, H., Scollon, S., Reuther, J., Voicu, H., Rednam, S. P., Lin, F. Y., Fisher, K. E., Chintagumpala, M., Adesina, A. M., Parsons, D. W., Plon, S. E., & Roy, A. (2019). Germline POLE mutation in a child with hypermutated medulloblastoma and features of constitutional mismatch repair deficiency. *Cold Spring Harb Mol Case Stud*, *5*(5).  
<https://doi.org/10.1101/mcs.a004499>
- Mandelker, D., Schmidt, R. J., Ankala, A., McDonald Gibson, K., Bowser, M., Sharma, H., Duffy, E., Hegde, M., Santani, A., Lebo, M., & Funke, B. (2016). Navigating highly homologous genes in a molecular diagnostic setting: a resource for clinical next-generation sequencing. *Genet Med*, *18*(12), 1282-1289.  
<https://doi.org/10.1038/gim.2016.58>
- Marin, F., Canet-Hermida, J., Bianchi, V., Chung, J., Wimmer, K., Foulkes, W., Perez-Alonso, V., Dominguez-Pinilla, N., Sabado, C., Vazquez-Gomez, F., Molines, A., Fioravanti, V., Carrasco, E., Stengs, L., Edwards, M., Negm, L., Das, A., Aronson, M., Pastor, A., . . . Pineda, M. (2024). A Validated Highly Sensitive Microsatellite Instability Assay Accurately Identifies Individuals Harboring Biallelic Germline PMS2 Pathogenic Variants in Constitutional Mismatch Repair Deficiency. *Clin Chem*, *70*(5), 737-746.  
<https://doi.org/10.1093/clinchem/hvae027>
- Mas-Ponte, D., McCullough, M., & Supek, F. (2022). Spectrum of DNA mismatch repair failures viewed through the lens of cancer genomics and implications for therapy. *Clin Sci (Lond)*, *136*(5), 383-404.  
<https://doi.org/10.1042/CS20210682>
- Matsumoto, T., Esaki, M., Fujisawa, R., Nakamura, S., Yao, T., & Iida, M. (2009). Chromoendoscopy, narrow-band imaging colonoscopy, and autofluorescence colonoscopy for detection of diminutive colorectal neoplasia in familial adenomatous polyposis. *Dis Colon Rectum*, *52*(6), 1160-1165.  
<https://doi.org/10.1007/DCR.0b013e31819ef6fe>
- Merino, D. M., McShane, L. M., Fabrizio, D., Funari, V., Chen, S. J., White, J. R., Wenz, P., Baden, J., Barrett, J. C., Chaudhary, R., Chen, L., Chen, W. S., Cheng, J. H., Cyanam, D., Dickey, J. S., Gupta, V., Hellmann, M., Helman, E., Li, Y., . . . Consortium, T. M. B. H. (2020). Establishing guidelines to harmonize tumor mutational burden (TMB): in silico assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of Cancer Research TMB Harmonization Project. *J Immunother Cancer*, *8*(1).  
<https://doi.org/10.1136/jitc-2019-000147>
- Meyers, M., Wagner, M. W., Hwang, H. S., Kinsella, T. J., & Boothman, D. A. (2001). Role of the hMLH1 DNA mismatch repair protein in fluoropyrimidine-mediated cell death and cell cycle responses. *Cancer Res*, *61*(13), 5193-5201.  
<https://www.ncbi.nlm.nih.gov/pubmed/11431359>

Michaeli, O., Ladany, H., Erez, A., Ben Shachar, S., Izraeli, S., Lidzbarsky, G., Basel-Salmon, L., Biskup, S., Maruvka, Y. E., Toledano, H., & Goldberg, Y. (2022). Di-genic inheritance of germline POLE and PMS2 pathogenic variants causes a unique condition associated with pediatric cancer predisposition. *Clin Genet*, *101*(4), 442-447.  
<https://doi.org/10.1111/cge.14106>

Michaeli, O., & Tabori, U. (2018). Pediatric High Grade Gliomas in the Context of Cancer Predisposition Syndromes. *J Korean Neurosurg Soc*, *61*(3), 319-332.  
<https://doi.org/10.3340/jkns.2018.0031>

Middha, S., Yaeger, R., Shia, J., Stadler, Z. K., King, S., Guercio, S., Paroder, V., Bates, D. D. B., Rana, S., Diaz, L. A., Jr., Saltz, L., Segal, N., Ladanyi, M., Zehir, A., & Hechtman, J. F. (2019). Majority of B2M-Mutant and -Deficient Colorectal Carcinomas Achieve Clinical Benefit From Immune Checkpoint Inhibitor Therapy and Are Microsatellite Instability-High. *JCO Precis Oncol*, *3*.  
<https://doi.org/10.1200/PO.18.00321>

Mishra, A. K., Achari, R. B., Zameer, L., Achari, G., Gehani, A., Roy, P., Sudhaman, S., Bianchi, V., Edwards, M., Sen, S., Sukumaran, R. K., Bhattacharyya, A., Tabori, U., & Das, A. (2022). Germline Biallelic Mismatch Repair Deficiency in Childhood Glioblastoma and Implications for Clinical Management. *Neurol India*, *70*(2), 772-774.  
<https://doi.org/10.4103/0028-3886.344608>

Mojtahed, A., Schrijver, I., Ford, J. M., Longacre, T. A., & Pai, R. K. (2011). A two-antibody mismatch repair protein immunohistochemistry screening approach for colorectal carcinomas, skin sebaceous tumors, and gynecologic tract carcinomas. *Mod Pathol*, *24*(7), 1004-1014.  
<https://doi.org/10.1038/modpathol.2011.55>

Moller, P., Seppala, T., Bernstein, I., Holinski-Feder, E., Sala, P., Evans, D. G., Lindblom, A., Macrae, F., Blanco, I., Sijmons, R., Jeffries, J., Vasen, H., Burn, J., Nakken, S., Hovig, E., Rodland, E. A., Tharmaratnam, K., de Vos Tot Nederveen Cappel, W. H., Hill, J., . . . Mallorca, G. (2017). Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. *Gut*, *66*(3), 464-472.  
<https://doi.org/10.1136/gutjnl-2015-309675>

Moller, P., Seppala, T. T., Bernstein, I., Holinski-Feder, E., Sala, P., Gareth Evans, D., Lindblom, A., Macrae, F., Blanco, I., Sijmons, R. H., Jeffries, J., Vasen, H. F. A., Burn, J., Nakken, S., Hovig, E., Rodland, E. A., Tharmaratnam, K., de Vos Tot Nederveen Cappel, W. H., Hill, J., . . . Mallorca, G. (2018). 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*, *67*(7), 1306-1316.  
<https://doi.org/10.1136/gutjnl-2017-314057>

Mork, M. E., You, Y. N., Ying, J., Bannon, S. A., Lynch, P. M., Rodriguez-Bigas, M. A., & Vilar, E. (2015). High Prevalence of Hereditary Cancer Syndromes in Adolescents and Young Adults With Colorectal Cancer. *J Clin Oncol*, *33*(31), 3544-3549.  
<https://doi.org/10.1200/JCO.2015.61.4503>

Negrini, S., Gorgoulis, V. G., & Halazonetis, T. D. (2010). Genomic instability--an evolving hallmark of cancer. *Nat Rev Mol Cell Biol*, *11*(3), 220-228.  
<https://doi.org/10.1038/nrm2858>

Ozyoruk, D., Cabi, E. U., Tacyildiz, N., Pinarli, F., Erdogan, A. O., Hanalioglu, S., Erdem, A. Y., & Demir, A. M. (2021). Cancer and constitutional Mismatch Repair Deficiency syndrome due to homozygous MSH 6 mutation in children with Cafe au Lait Spots and review of literature. *Turk J Pediatr*, *63*(5), 893-902.  
<https://doi.org/10.24953/turkjped.2021.05.017>

Paul, C., Chakraborty, S., Chakraborty, S., & Goswami, K. (2020). Constitutional Mismatch Repair Deficiency Syndrome in a patient from India. *Clin Case Rep*, *8*(12), 2824-2826.  
<https://doi.org/10.1002/ccr3.3249>

Pavelka, Z., Zitterbart, K., Noskova, H., Bajciova, V., Slaby, O., & Sterba, J. (2019). Effective Immunotherapy of Glioblastoma in an Adolescent with Constitutional Mismatch Repair-Deficiency Syndrome. *Klin Onkol*, 32(1), 70-74.  
<https://doi.org/10.14735/amko201970> (U&#269;inna imunoterapie glioblastomu u adolescenta se syndromem konstitu&#269;niho deficitu v mismatch repair opravnem systemu.)

Perez-Valencia, J. A., Gallon, R., Chen, Y., Koch, J., Keller, M., Oberhuber, K., Gomes, A., Zschocke, J., Burn, J., Jackson, M. S., Santibanez-Koref, M., Messiaen, L., & Wimmer, K. (2020). Constitutional mismatch repair deficiency is the diagnosis in 0.41% of pathogenic NF1/SPRED1 variant negative children suspected of sporadic neurofibromatosis type 1. *Genet Med*, 22(12), 2081-2088.  
<https://doi.org/10.1038/s41436-020-0925-z>

Peron, S., Metin, A., Gardes, P., Alyanakian, M. A., Sheridan, E., Kratz, C. P., Fischer, A., & Durandy, A. (2008). Human PMS2 deficiency is associated with impaired immunoglobulin class switch recombination. *J Exp Med*, 205(11), 2465-2472.  
<https://doi.org/10.1084/jem.20080789>

Perrod, G., Rahmi, G., & Cellier, C. (2021). Colorectal cancer screening in Lynch syndrome: Indication, techniques and future perspectives. *Dig Endosc*, 33(4), 520-528.  
<https://doi.org/10.1111/den.13702>

Phelps, R., Gallon, R., Hayes, C., Glover, E., Gibson, P., Edidi, I., Lee, T., Mills, S., Shaw, A., Heer, R., Ralte, A., McNulty, C., Santibanez-Koref, M., Burn, J., & Jackson, M. S. (2022). Detection of Microsatellite Instability in Colonoscopic Biopsies and Postal Urine Samples from Lynch Syndrome Cancer Patients Using a Multiplex PCR Assay. *Cancers (Basel)*, 14(15).  
<https://doi.org/10.3390/cancers14153838>

Pollack, I. F., Hamilton, R. L., Sobol, R. W., Nikiforova, M. N., Nikiforov, Y. E., Lyons-Weiler, M. A., LaFramboise, W. A., Burger, P. C., Brat, D. J., Rosenblum, M. K., Gilles, F. H., Yates, A. J., Zhou, T., Cohen, K. J., Finlay, J. L., Jakacki, R. I., & Children's Oncology, G. (2010). Mismatch repair deficiency is an uncommon mechanism of alkylator resistance in pediatric malignant gliomas: a report from the Children's Oncology Group. *Pediatr Blood Cancer*, 55(6), 1066-1071.  
<https://doi.org/10.1002/pbc.22634>

Porter, C. C., Druley, T. E., Erez, A., Kuiper, R. P., Onel, K., Schiffman, J. D., Wolfe Schneider, K., Scollon, S. R., Scott, H. S., Strong, L. C., Walsh, M. F., & Nichols, K. E. (2017). Recommendations for Surveillance for Children with Leukemia-Predisposing Conditions. *Clin Cancer Res*, 23(11), e14-e22.  
<https://doi.org/10.1158/1078-0432.CCR-17-0428>

Quinn, E., & Nichols, K. E. (2017). Cancer predisposition syndromes associated with myeloid malignancy. *Semin Hematol*, 54(2), 115-122.  
<https://doi.org/10.1053/j.seminhematol.2017.04.003>

Ramchander, N. C., Ryan, N. A., Crosbie, E. J., & Evans, D. G. (2017). Homozygous germ-line mutation of the PMS2 mismatch repair gene: a unique case report of constitutional mismatch repair deficiency (CMMRD). *BMC Med Genet*, 18(1), 40.  
<https://doi.org/10.1186/s12881-017-0391-x>

Raveneau, M., Guerrini-Rousseau, L., Levy, R., Roux, C. J., Bolle, S., Doz, F., Bourdeaut, F., Colas, C., Blauwblomme, T., Beccaria, K., Tauziede-Espariat, A., Varlet, P., Dufour, C., Grill, J., Boddaert, N., & Dangouloff-Ros, V. (2024). Specific brain MRI features of constitutional mismatch repair deficiency syndrome in children with high-grade gliomas. *Eur Radiol*.  
<https://doi.org/10.1007/s00330-024-10885-3>

Ricciardone, M. D., Ozcelik, T., Cevher, B., Ozdag, H., Tuncer, M., Gurgey, A., Uzunalimoglu, O., Cetinkaya, H., Tanyeli, A., Erken, E., & Ozturk, M. (1999). Human MLH1 deficiency predisposes to hematological malignancy and neurofibromatosis type 1. *Cancer Res*, 59(2), 290-293.  
<https://www.ncbi.nlm.nih.gov/pubmed/9927033>

Rigaud, C., Forster, V. J., Al-Tarrah, H., Attarbaschi, A., Bianchi, V., Burke, A., Burkhardt, B., Colas, C., Devalck, C., Edwards, M., Elitzur, S., Garthe, A. K., Goldberg, Y., Guerrini-Rousseau, L.,

Horpaopan, S., Januszkiewicz-Lewandowska, D., Kabickova, E., Kratz, C. P., Loeffen, J., . . . Brugieres, L. (2024). Comprehensive analysis of constitutional mismatch repair deficiency-associated non-Hodgkin lymphomas in a global cohort. *Pediatr Blood Cancer*, e31302.  
<https://doi.org/10.1002/pbc.31302>

Ripperger, T., Beger, C., Rahner, N., Sykora, K. W., Bockmeyer, C. L., Lehmann, U., Kreipe, H. H., & Schlegelberger, B. (2010). Constitutional mismatch repair deficiency and childhood leukemia/lymphoma--report on a novel biallelic MSH6 mutation. *Haematologica*, 95(5), 841-844.  
<https://doi.org/10.3324/haematol.2009.015503>

Ripperger, T., Evans, D. G., Malkin, D., & Kratz, C. P. (2021). Choose and stay on one out of two paths: distinction between clinical versus research genetic testing to identify cancer predisposition syndromes among patients with cancer. *Fam Cancer*, 20(4), 289-291.  
<https://doi.org/10.1007/s10689-021-00228-2>

Ripperger, T., & Schlegelberger, B. (2016). Acute lymphoblastic leukemia and lymphoma in the context of constitutional mismatch repair deficiency syndrome. *Eur J Med Genet*, 59(3), 133-142.  
<https://doi.org/10.1016/j.ejmg.2015.12.014>

Rittberg, R., Harlos, C., Rothenmund, H., Das, A., Tabori, U., Sinha, N., Singh, H., Chodirker, B., & Kim, C. A. (2021). Immune Checkpoint Inhibition as Primary Adjuvant Therapy for an IDH1-Mutant Anaplastic Astrocytoma in a Patient with CMMRD: A Case Report-Usage of Immune Checkpoint Inhibition in CMMRD. *Curr Oncol*, 28(1), 757-766.  
<https://doi.org/10.3390/curroncol28010074>

Sahebjam, S., Forsyth, P. A., Tran, N. D., Arrington, J. A., Macaulay, R., Etame, A. B., Walko, C. M., Boyle, T., Peguero, E. N., Jaglal, M., Mokhtari, S., Enderling, H., Raghunand, N., Gatewood, T., Long, W., Dzierzeski, J. L., Evernden, B., Robinson, T., Wicklund, M. C., . . . Yu, H. M. (2021). Hypofractionated stereotactic re-irradiation with pembrolizumab and bevacizumab in patients with recurrent high-grade gliomas: results from a phase I study. *Neuro Oncol*, 23(4), 677-686.  
<https://doi.org/10.1093/neuonc/noaa260>

Sahin, I. H., Akce, M., Alese, O., Shaib, W., Lesinski, G. B., El-Rayes, B., & Wu, C. (2019). Immune checkpoint inhibitors for the treatment of MSI-H/MMR-D colorectal cancer and a perspective on resistance mechanisms. *Br J Cancer*, 121(10), 809-818.  
<https://doi.org/10.1038/s41416-019-0599-y>

Sawant, A., Kothandapani, A., Zhitkovich, A., Sobol, R. W., & Patrick, S. M. (2015). Role of mismatch repair proteins in the processing of cisplatin interstrand cross-links. *DNA Repair (Amst)*, 35, 126-136.  
<https://doi.org/10.1016/j.dnarep.2015.10.003>

Schamschula, E., Kinzel, M., Wernstedt, A., Oberhuber, K., Gottschling, H., Schnaiter, S., Friedrichs, N., Merkelbach-Bruse, S., Zschocke, J., Gallon, R., & Wimmer, K. (2022). Teenage-Onset Colorectal Cancers in a Digenic Cancer Predisposition Syndrome Provide Clues for the Interaction between Mismatch Repair and Polymerase delta Proofreading Deficiency in Tumorigenesis. *Biomolecules*, 12(10).  
<https://doi.org/10.3390/biom12101350>

Schrock, A. B., Ouyang, C., Sandhu, J., Sokol, E., Jin, D., Ross, J. S., Miller, V. A., Lim, D., Amanam, I., Chao, J., Catenacci, D., Cho, M., Braiteh, F., Klempner, S. J., Ali, S. M., & Fakih, M. (2019). Tumor mutational burden is predictive of response to immune checkpoint inhibitors in MSI-high metastatic colorectal cancer. *Ann Oncol*, 30(7), 1096-1103.  
<https://doi.org/10.1093/annonc/mdz134>

Scott, R. H., Homfray, T., Huxter, N. L., Mitton, S. G., Nash, R., Potter, M. N., Lancaster, D., & Rahman, N. (2007). Familial T-cell non-Hodgkin lymphoma caused by biallelic MSH2 mutations. *J Med Genet*, 44(7), e83.  
<https://doi.org/10.1136/jmg.2007.048942>

Sehested, A., Meade, J., Scheie, D., Ostrup, O., Bertelsen, B., Misiakou, M. A., Sarosiek, T., Kessler, E., Melchior, L. C., Munch-Petersen, H. F., Pai, R. K., Schmuth, M., Gottschling, H., Zschocke, J.,



Gallon, R., & Wimmer, K. (2022). Constitutional POLE variants causing a phenotype reminiscent of constitutional mismatch repair deficiency. *Hum Mutat*, 43(1), 85-96.  
<https://doi.org/10.1002/humu.24299>

Sekine, S., Mori, T., Ogawa, R., Tanaka, M., Yoshida, H., Taniguchi, H., Nakajima, T., Sugano, K., Yoshida, T., Kato, M., Furukawa, E., Ochiai, A., & Hiraoka, N. (2017). Mismatch repair deficiency commonly precedes adenoma formation in Lynch Syndrome-Associated colorectal tumorigenesis. *Mod Pathol*, 30(8), 1144-1151.  
<https://doi.org/10.1038/modpathol.2017.39>

Serrano, D., Patrignani, P., Stigliano, V., Turchetti, D., Sciallero, S., Roviello, F., D'Arpino, A., Grattagliano, I., Testa, S., Oliani, C., Bertario, L., & Bonanni, B. (2022). Aspirin Colorectal Cancer Prevention in Lynch Syndrome: Recommendations in the Era of Precision Medicine. *Genes (Basel)*, 13(3).  
<https://doi.org/10.3390/genes13030460>

Sha, D., Jin, Z., Budczies, J., Kluck, K., Stenzinger, A., & Sinicrope, F. A. (2020). Tumor Mutational Burden as a Predictive Biomarker in Solid Tumors. *Cancer Discov*, 10(12), 1808-1825.  
<https://doi.org/10.1158/2159-8290.CD-20-0522>

Shimamura, Y., Walsh, C. M., Cohen, S., Aronson, M., Tabori, U., Kortan, P. P., Durno, C. A., & the International, B. C. (2018). Role of video capsule endoscopy in patients with constitutional mismatch repair deficiency (CMMRD) syndrome: report from the International CMMRD Consortium. *Endosc Int Open*, 6(8), E1037-E1043.  
<https://doi.org/10.1055/a-0591-9054>

Shiran, S. I., Ben-Sira, L., Elhasid, R., Roth, J., Tabori, U., Yalon, M., Constantini, S., & Dvir, R. (2018). Multiple Brain Developmental Venous Anomalies as a Marker for Constitutional Mismatch Repair Deficiency Syndrome. *AJNR Am J Neuroradiol*, 39(10), 1943-1946.  
<https://doi.org/10.3174/ajnr.A5766>

Shlien, A., Campbell, B. B., de Borja, R., Alexandrov, L. B., Merico, D., Wedge, D., Van Loo, P., Tarpey, P. S., Coupland, P., Behjati, S., Pollett, A., Lipman, T., Heidari, A., Deshmukh, S., Avitzur, N., Meier, B., Gerstung, M., Hong, Y., Merino, D. M., . . . Biallelic Mismatch Repair Deficiency, C. (2015). Combined hereditary and somatic mutations of replication error repair genes result in rapid onset of ultra-hypermutated cancers. *Nat Genet*, 47(3), 257-262.  
<https://doi.org/10.1038/ng.3202>

Shuen, A. Y., Lanni, S., Panigrahi, G. B., Edwards, M., Yu, L., Campbell, B. B., Mandel, A., Zhang, C., Zhukova, N., Alharbi, M., Bernstein, M., Bowers, D. C., Carroll, S., Cole, K. A., Constantini, S., Crooks, B., Dvir, R., Farah, R., Hijiya, N., . . . Pearson, C. E. (2019). Functional Repair Assay for the Diagnosis of Constitutional Mismatch Repair Deficiency From Non-Neoplastic Tissue. *J Clin Oncol*, 37(6), 461-470.  
<https://doi.org/10.1200/JCO.18.00474>

Southey, M. C., Jenkins, M. A., Mead, L., Whitty, J., Trivett, M., Tesoriero, A. A., Smith, L. D., Jennings, K., Grubb, G., Royce, S. G., Walsh, M. D., Barker, M. A., Young, J. P., Jass, J. R., St John, D. J., Macrae, F. A., Giles, G. G., & Hopper, J. L. (2005). Use of molecular tumor characteristics to prioritize mismatch repair gene testing in early-onset colorectal cancer. *J Clin Oncol*, 23(27), 6524-6532.  
<https://doi.org/10.1200/JCO.2005.04.671>

Stoffel, E. M., Mangu, P. B., Gruber, S. B., Hamilton, S. R., Kalady, M. F., Lau, M. W., Lu, K. H., Roach, N., Limburg, P. J., American Society of Clinical, O., & European Society of Clinical, O. (2015). 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*, 33(2), 209-217.  
<https://doi.org/10.1200/JCO.2014.58.1322>

Stojic, L., Brun, R., & Jiricny, J. (2004). Mismatch repair and DNA damage signalling. *DNA Repair (Amst)*, 3(8-9), 1091-1101.  
<https://doi.org/10.1016/j.dnarep.2004.06.006>

Suerink, M., Kilinc, G., Terlouw, D., Hristova, H., Sensuk, L., van Egmond, D., Farina Sarasqueta, A., Langers, A. M. J., van Wezel, T., Morreau, H., Nielsen, M., & collaborators, P. A.-g. (2021a). Prevalence of mismatch repair deficiency and Lynch syndrome in a cohort of unselected small bowel adenocarcinomas. *J Clin Pathol*, 74(11), 724-729.  
<https://doi.org/10.1136/jclinpath-2020-207040>

Suerink, M., Potjer, T. P., Versluijs, A. B., Ten Broeke, S. W., Tops, C. M., Wimmer, K., & Nielsen, M. (2018). Constitutional mismatch repair deficiency in a healthy child: On the spot diagnosis? *Clin Genet*, 93(1), 134-137.  
<https://doi.org/10.1111/cge.13053>

Suerink, M., Ripperger, T., Messiaen, L., Menko, F. H., Bourdeaut, F., Colas, C., Jongmans, M., Goldberg, Y., Nielsen, M., Muleris, M., van Kouwen, M., Slavc, I., Kratz, C., Vasen, H. F., Brugières, L., Legius, E., & Wimmer, K. (2019a). Constitutional mismatch repair deficiency as a differential diagnosis of neurofibromatosis type 1: consensus guidelines for testing a child without malignancy. *J Med Genet*, 56(2), 53-62.  
<https://doi.org/10.1136/jmedgenet-2018-105664>

Suerink, M., Rodriguez-Gironde, M., van der Klift, H. M., Colas, C., Brugières, L., Lavoine, N., Jongmans, M., Munar, G. C., Evans, D. G., Farrell, M. P., Genuardi, M., Goldberg, Y., Gomez-Garcia, E., Heinemann, K., Hoell, J. I., Aretz, S., Jaspersen, K. W., Kedar, I., Modi, M. B., . . . Nielsen, M. (2019b). An alternative approach to establishing unbiased colorectal cancer risk estimation in Lynch syndrome. *Genet Med*, 21(12), 2706-2712.  
<https://doi.org/10.1038/s41436-019-0577-z>

Suerink, M., Wimmer, K., Brugières, L., Colas, C., Gallon, R., Ripperger, T., Benusiglio, P. R., Bleiker, E. M. A., Ghorbanoghli, Z., Goldberg, Y., Hardwick, J. C. H., Kloor, M., le Mentec, M., Muleris, M., Pineda, M., Ruiz-Ponte, C., & Vasen, H. F. A. (2021b). Report of the fifth meeting of the European Consortium 'Care for CMMRD' (C4CMMRD), Leiden, The Netherlands, July 6th 2019. *Fam Cancer*, 20(1), 67-73.  
<https://doi.org/10.1007/s10689-020-00194-1>

Tabori, U., Hansford, J. R., Achatz, M. I., Kratz, C. P., Plon, S. E., Frebourg, T., & Brugières, L. (2017). Clinical Management and Tumor Surveillance Recommendations of Inherited Mismatch Repair Deficiency in Childhood. *Clin Cancer Res*, 23(11), e32-e37.  
<https://doi.org/10.1158/1078-0432.CCR-17-0574>

Tanimura, K., Yamasaki, K., Matsubara, K., Noguchi, M., Kikuchi, N., Nitani, C., Okada, K., Fujisaki, H., Okuno, T., Inoue, T., Nebiki, H., Akagi, K., Tomita, N., & Hara, J. (2022). Could the immune checkpoint inhibitor against colorectal cancer in constitutional mismatch repair deficiency syndrome prevent new cancer formation? *Pediatr Blood Cancer*, 69(1), e29312.  
<https://doi.org/10.1002/pbc.29312>

Ten Broeke, S. W., van der Klift, H. M., Tops, C. M. J., Aretz, S., Bernstein, I., Buchanan, D. D., de la Chapelle, A., Capella, G., Clendenning, M., Engel, C., Gallinger, S., Gomez Garcia, E., Figueiredo, J. C., Haile, R., Hampel, H. L., Hopper, J. L., Hoogerbrugge, N., von Knebel Doeberitz, M., Le Marchand, L., . . . Win, A. K. (2018). Cancer Risks for PMS2-Associated Lynch Syndrome. *J Clin Oncol*, 36(29), 2961-2968.  
<https://doi.org/10.1200/JCO.2018.78.4777>

Tesch, V. K., H, I. J., Raicht, A., Rueda, D., Dominguez-Pinilla, N., Allende, L. M., Colas, C., Rosenbaum, T., Ilencikova, D., Baris, H. N., Nathrath, M. H. M., Suerink, M., Januszkiewicz-Lewandowska, D., Ragab, I., Azizi, A. A., Wenzel, S. S., Zschocke, J., Schwinger, W., Kloor, M., . . . Seidel, M. G. (2018). No Overt Clinical Immunodeficiency Despite Immune Biological Abnormalities in Patients With Constitutional Mismatch Repair Deficiency. *Front Immunol*, 9, 1506.

<https://doi.org/10.3389/fimmu.2018.01506>

Tewattanarat, N., Junhasavasdikul, T., Panwar, S., Joshi, S. D., Abadeh, A., Greer, M. L. C., Goldenberg, A., Zheng, G., Villani, A., Malkin, D., & Doria, A. S. (2022). Diagnostic accuracy of imaging approaches for early tumor detection in children with Li-Fraumeni syndrome. *Pediatr Radiol*, 52(7), 1283-1295.

<https://doi.org/10.1007/s00247-022-05296-9>

Thatikonda, V., Islam, S. M. A., Autry, R. J., Jones, B. C., Grobner, S. N., Warsow, G., Hutter, B., Huebschmann, D., Frohling, S., Kool, M., Blattner-Johnson, M., Jones, D. T. W., Alexandrov, L. B., Pfister, S. M., & Jager, N. (2023). Comprehensive analysis of mutational signatures reveals distinct patterns and molecular processes across 27 pediatric cancers. *Nat Cancer*, 4(2), 276-289.

<https://doi.org/10.1038/s43018-022-00509-4>

Therkildsen, C., Jensen, L. H., Rasmussen, M., & Bernstein, I. (2021). An Update on Immune Checkpoint Therapy for the Treatment of Lynch Syndrome. *Clin Exp Gastroenterol*, 14, 181-197.

<https://doi.org/10.2147/CEG.S278054>

Toledano, H., Orenstein, N., Sofrin, E., Ruhrman-Shahar, N., Amarilyo, G., Basel-Salmon, L., Shuldiner, A. R., Smirin-Yosef, P., Aronson, M., Al-Tarrah, H., Bazak, L., Gonzaga-Jauregui, C., Tabori, U., Wimmer, K., & Goldberg, Y. (2020). Paediatric systemic lupus erythematosus as a manifestation of constitutional mismatch repair deficiency. *J Med Genet*, 57(7), 505-508.

<https://doi.org/10.1136/jmedgenet-2019-106303>

Tomasello, G., Ghidini, M., Galassi, B., Grossi, F., Luciani, A., & Petrelli, F. (2022). Survival benefit with adjuvant chemotherapy in stage III microsatellite-high/deficient mismatch repair colon cancer: a systematic review and meta-analysis. *Sci Rep*, 12(1), 1055.

<https://doi.org/10.1038/s41598-022-05065-6>

Touat, M., Li, Y. Y., Boynton, A. N., Spurr, L. F., Iorgulescu, J. B., Bohrsen, C. L., Cortes-Ciriano, I., Birzu, C., Geduldig, J. E., Pelton, K., Lim-Fat, M. J., Pal, S., Ferrer-Luna, R., Ramkissoon, S. H., Dubois, F., Bellamy, C., Currimjee, N., Bonardi, J., Qian, K., . . . Ligon, K. L. (2020). Mechanisms and therapeutic implications of hypermutation in gliomas. *Nature*, 580(7804), 517-523.

<https://doi.org/10.1038/s41586-020-2209-9>

Trimbath, J. D., Petersen, G. M., Erdman, S. H., Ferre, M., Luce, M. C., & Giardiello, F. M. (2001). Cafe-au-lait spots and early onset colorectal neoplasia: a variant of HNPCC? *Fam Cancer*, 1(2), 101-105.

<https://doi.org/10.1023/a:1013881832014>

Turcot, J., Despres, J. P., & St Pierre, F. (1959). Malignant tumors of the central nervous system associated with familial polyposis of the colon: report of two cases. *Dis Colon Rectum*, 2, 465-468.

<https://doi.org/10.1007/BF02616938>

Urganci, N., Genc, D. B., Kose, G., Onal, Z., & Vidin, O. O. (2015). Colorectal Cancer due to Constitutional Mismatch Repair Deficiency Mimicking Neurofibromatosis I. *Pediatrics*, 136(4), e1047-1050.

<https://doi.org/10.1542/peds.2015-1426>

van der Klift, H. M., Mensenkamp, A. R., Drost, M., Bik, E. C., Vos, Y. J., Gille, H. J., Redeker, B. E., Tiersma, Y., Zonneveld, J. B., Garcia, E. G., Letteboer, T. G., Olderde-Berends, M. J., van Hest, L. P., van Os, T. A., Verhoef, S., Wagner, A., van Asperen, C. J., Ten Broeke, S. W., Hes, F. J., . . . Tops, C. M. (2016). Comprehensive Mutation Analysis of PMS2 in a Large Cohort of Proband Suspected of Lynch Syndrome or Constitutional Mismatch Repair Deficiency Syndrome. *Hum Mutat*, 37(11), 1162-1179.

<https://doi.org/10.1002/humu.23052>

van der Klift, H. M., Tops, C. M., Bik, E. C., Boogaard, M. W., Borgstein, A. M., Hansson, K. B., Ausems, M. G., Gomez Garcia, E., Green, A., Hes, F. J., Izatt, L., van Hest, L. P., Alonso, A. M., Vriends, A. H., Wagner, A., van Zelst-Stams, W. A., Vasen, H. F., Morreau, H., Devilee, P., & Wijnen, J. T. (2010). Quantification of sequence exchange events between PMS2 and PMS2CL provides a basis for improved mutation scanning of Lynch syndrome patients. *Hum Mutat*, 31(5), 578-587.

<https://doi.org/10.1002/humu.21229>

van Leerdam, M. E., Roos, V. H., van Hooft, J. E., Balaguer, F., Dekker, E., Kaminski, M. F., Latchford, A., Neumann, H., Ricciardiello, L., Rupinska, M., Saurin, J. C., Tanis, P. J., Wagner, A., Jover, R., & Pellise, M. (2019). Endoscopic management of Lynch syndrome and of familial risk of colorectal cancer: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy*, *51*(11), 1082-1093.  
<https://doi.org/10.1055/a-1016-4977>

Vasen, H. F., Ghorbanoghli, Z., Bourdeaut, F., Cabaret, O., Caron, O., Duval, A., Entz-Werle, N., Goldberg, Y., Ilencikova, D., Kratz, C. P., Lavoine, N., Loeffen, J., Menko, F. H., Muleris, M., Sebille, G., Colas, C., Burkhardt, B., Brugieres, L., Wimmer, K., & CMMR-D, E. U.-C. C. f. (2014). Guidelines for surveillance of individuals with constitutional mismatch repair-deficiency proposed by the European Consortium "Care for CMMR-D" (C4CMMR-D). *J Med Genet*, *51*(5), 283-293.  
<https://doi.org/10.1136/jmedgenet-2013-102238>

Vasantachart, J. M., Zacher, N. C., & Teng, J. M. C. (2022). Eruptive nevi in a patient with constitutional mismatch repair deficiency (CMMRD). *Pediatr Dermatol*, *39*(1), 91-93.  
<https://doi.org/10.1111/pde.14861>

Vaughn, C. P., Hart, K. J., Samowitz, W. S., & Swensen, J. J. (2011). Avoidance of pseudogene interference in the detection of 3' deletions in PMS2. *Hum Mutat*, *32*(9), 1063-1071.  
<https://doi.org/10.1002/humu.21540>

Vaughn, C. P., Robles, J., Swensen, J. J., Miller, C. E., Lyon, E., Mao, R., Bayrak-Toydemir, P., & Samowitz, W. S. (2010). Clinical analysis of PMS2: mutation detection and avoidance of pseudogenes. *Hum Mutat*, *31*(5), 588-593.  
<https://doi.org/10.1002/humu.21230>

Wang, Q., Lasset, C., Desseigne, F., Frappaz, D., Bergeron, C., Navarro, C., Ruano, E., & Puisieux, A. (1999). Neurofibromatosis and early onset of cancers in hMLH1-deficient children. *Cancer Res*, *59*(2), 294-297.  
<https://www.ncbi.nlm.nih.gov/pubmed/9927034>

Ward, D. G., Baxter, L., Ott, S., Gordon, N. S., Wang, J., Patel, P., Piechocki, K., Silcock, L., Sale, C., Zeegers, M. P., Cheng, K. K., James, N. D., Bryan, R. T., & BladderPath Trial Management, G. (2023). Highly Sensitive and Specific Detection of Bladder Cancer via Targeted Ultra-deep Sequencing of Urinary DNA. *Eur Urol Oncol*, *6*(1), 67-75.  
<https://doi.org/10.1016/j.euo.2022.03.005>

Waterfall, J. J., & Meltzer, P. S. (2015). Avalanching mutations in biallelic mismatch repair deficiency syndrome. *Nat Genet*, *47*(3), 194-196.  
<https://doi.org/10.1038/ng.3227>

Watson, P., Vasen, H. F. A., Mecklin, J. P., Bernstein, I., Aarnio, M., Jarvinen, H. J., Myrhoj, T., Sunde, L., Wijnen, J. T., & Lynch, H. T. (2008). The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. *Int J Cancer*, *123*(2), 444-449.  
<https://doi.org/10.1002/ijc.23508>

Wernstedt, A., Valtorta, E., Armelao, F., Togni, R., Girlando, S., Baudis, M., Heinemann, K., Messiaen, L., Staehli, N., Zschocke, J., Marra, G., & Wimmer, K. (2012). Improved multiplex ligation-dependent probe amplification analysis identifies a deleterious PMS2 allele generated by recombination with crossover between PMS2 and PMS2CL. *Genes Chromosomes Cancer*, *51*(9), 819-831.  
<https://doi.org/10.1002/gcc.21966>

Westdorp, H., Kolders, S., Hoogerbrugge, N., de Vries, I. J. M., Jongmans, M. C. J., & Schreiber, G. (2017). Immunotherapy holds the key to cancer treatment and prevention in constitutional mismatch repair deficiency (CMMRD) syndrome. *Cancer Lett*, *403*, 159-164.  
<https://doi.org/10.1016/j.canlet.2017.06.018>

Wimmer, K., Beilken, A., Nustede, R., Ripberger, T., Lamottke, B., Ure, B., Steinmann, D., Reineke-Plaass, T., Lehmann, U., Zschocke, J., Valle, L., Fauth, C., & Kratz, C. P. (2017a). A novel germline

POLE mutation causes an early onset cancer prone syndrome mimicking constitutional mismatch repair deficiency. *Fam Cancer*, 16(1), 67-71.

<https://doi.org/10.1007/s10689-016-9925-1>

Wimmer, K., Kratz, C. P., Vasen, H. F., Caron, O., Colas, C., Entz-Werle, N., Gerdes, A. M., Goldberg, Y., Ilencikova, D., Muleris, M., Duval, A., Lavoine, N., Ruiz-Ponte, C., Slavic, I., Burkhardt, B., Brugieres, L., & CMMRD, E. U.-C. C. f. (2014). Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'care for CMMRD' (C4CMMRD). *J Med Genet*, 51(6), 355-365.

<https://doi.org/10.1136/jmedgenet-2014-102284>

Wimmer, K., Rosenbaum, T., & Messiaen, L. (2017b). Connections between constitutional mismatch repair deficiency syndrome and neurofibromatosis type 1. *Clin Genet*, 91(4), 507-519.

<https://doi.org/10.1111/cge.12904>

Win, A. K., Jenkins, M. A., Dowty, J. G., Antoniou, A. C., Lee, A., Giles, G. G., Buchanan, D. D., Clendenning, M., Rosty, C., Ahnen, D. J., Thibodeau, S. N., Casey, G., Gallinger, S., Le Marchand, L., Haile, R. W., Potter, J. D., Zheng, Y., Lindor, N. M., Newcomb, P. A., . . . MacInnis, R. J. (2017). Prevalence and Penetrance of Major Genes and Polygenes for Colorectal Cancer. *Cancer Epidemiol Biomarkers Prev*, 26(3), 404-412.

<https://doi.org/10.1158/1055-9965.EPI-16-0693>

Xiao, Y., Ju, L., Qian, K., Jin, W., Wang, G., Zhao, Y., Jiang, W., Liu, N., Wu, K., Peng, M., Cao, R., Li, S., Shi, H., Gong, Y., Zheng, H., Liu, T., Luo, Y., Ma, H., Chang, L., . . . Wang, X. (2022). Non-invasive diagnosis and surveillance of bladder cancer with driver and passenger DNA methylation in a prospective cohort study. *Clin Transl Med*, 12(8), e1008.

<https://doi.org/10.1002/ctm2.1008>

Xie, T., Feng, Q., Li, Z., Lu, M., Li, J., Lizaso, A., Xiang, J., Zhang, L., Shen, L., & Peng, Z. (2021). Heterogeneous constitutional mismatch repair deficiency with MSH6 missense mutation clinically benefits from pembrolizumab and regorafenib combination therapy: a case report and literature review. *Hered Cancer Clin Pract*, 19(1), 7.

<https://doi.org/10.1186/s13053-021-00165-2>

Zou, X., Koh, G. C. C., Nanda, A. S., Degasperis, A., Urgo, K., Roumeliotis, T. I., Agu, C. A., Badja, C., Momen, S., Young, J., Amarante, T. D., Side, L., Brice, G., Perez-Alonso, V., Rueda, D., Gomez, C., Bushell, W., Harris, R., Choudhary, J. S., . . . Nik-Zainal, S. (2021). A systematic CRISPR screen defines mutational mechanisms underpinning signatures caused by replication errors and endogenous DNA damage. *Nat Cancer*, 2(6), 643-657.

<https://doi.org/10.1038/s43018-021-00200-0>