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**Guidelines for the diagnosis of Constitutional Mismatch Repair Deficiency (CMMRD);
Recommendations from the international consensus working group.**

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**** Abstract – to be written based on journal**

Introduction

Constitutional Mismatch Repair Deficiency Syndrome (CMMRD), caused by biallelic pathogenic variants (PV) in mismatch repair (MMR) genes, is one of the most aggressive and complex hereditary cancer syndrome with a very high prevalence of synchronous and metachronous cancers resulting in patient death in early life. CMMRD can also present with benign manifestations resembling neurofibromatosis type I (NF1).¹ While most malignancies present in childhood, there is variability in the age and presentation of cancers and benign manifestations.

Of the 4 MMR genes, *PMS2* gene is responsible for over 60% of patients with CMMRD, followed by *MSH6* (20-30%), *MLH1* and *MSH2* (10-20%).²⁻⁴ Consanguinity has been reported in 39-45% of CMMRD families, varying slightly depending on country of origin.³⁻⁶ Homozygous variants have been reported in 10-28.5% of families that deny consanguinity suggesting common founder mutations, with higher frequency in isolated populations or those that may share a common ancestor.^{3, 4, 6, 7}

Genetic testing for CMMRD may not be definitive as the most commonly impacted gene, *PMS2*, can pose testing challenges due to pseudogenes and frequent gene conversion events that can be circumvented only by specialized assays.^{8, 9} Unclassified variants, or variants of uncertain significance (VUS), pose challenges for interpretation, and *PMS2* has the highest incidence of VUS among the MMR genes, comprising 49% of reported variants in this gene.¹⁰ Lack of definitive genetic confirmation in some individuals and a complex presentation of the disorder necessitates the need for diagnostic criteria. Together, the growing number of patients with clinical or tumor characteristics suggestive of CMMRD requires a robust pathway for genetic diagnosis and testing which allows for counselling, surveillance, and better tumor management.

An understanding of CMMRD requires background information on Lynch syndrome (LS), caused by monoallelic PV in the MMR genes. LS is an autosomal dominant condition predisposing primarily to adult-onset colorectal and endometrial cancer. Compared to CMMRD, there is an inverse distribution of gene mutations, with *MSH2* and *MLH1* accounting for 80% of families with LS, *MSH6* in 13% and *PMS2* in 6% of families.¹¹ In addition, deletions of the terminal exons of the *EPCAM* gene that include 3'UTR sequences upstream from *MSH2* cause methylation of *MSH2* leading to a LS phenotype.¹²⁻¹⁵

LS is associated with a high cancer penetrance leading to a strong family history of colorectal and endometrial cancer. *PMS2* and *MSH6* have a lower penetrance of CRC and endometrial cancer (10% and 12% in *PMS2* and 20% and 40% in *MSH6*, respectively),¹¹ which is supported by lack of family history for individuals with *PMS2* or *MSH6* related LS.^{1,4,6}

On the other hand, patients with CMMRD typically present in childhood with brain, lymphoma or GI cancers, although there is a broad spectrum of malignancies, including individuals presenting without cancer and/or NF1 manifestations. Given the range in phenotypical presentation, criteria for risk assessment and determining the need for genetic testing for CMMRD in pediatric or young adult patients were outlined by the European consortium CARE for CMMRD (C4CMMRD) (2014). This scoring system is based on a calculated clinical score of malignancy and benign features (Figure 1).¹ The C4CMMRD consortium also defined CMMRD counseling and testing criteria for children suspected to have sporadic NF1 but without a detectable mutation in *NF1* or *SPRED1* and without a malignancy.¹⁶

These scoring systems are helpful in identifying patients who should undergo genetic testing for CMMRD and are hence an entry point for evaluation in an individual. However, no diagnostic criteria currently encompass molecular results, ancillary testing and clinical presentation. Given the lack of one definitive test for CMMRD in most cases and the variable presentation, an international committee was convened to establish diagnostic criteria for CMMRD which are summarized in this paper. An accurate diagnosis has important implications for genetic counselling, tumor surveillance,^{2, 17, 18} (Table 1) and access to immunotherapy cancer treatment.^{19,20}

Methods

In order to establish diagnostic criteria, an international working group was formed, including 13 experts, with representatives from the International Replication Repair Deficiency Consortium (IRRD), the C4CMMRD (European consortium, Care for CMMRD) as well as other clinical and molecular geneticists, genetic counsellors, pediatric hematologist and oncologists, from Canada, United States, Austria, Israel and France.

To validate some of the recommendations, the clinical working group used data from the IRRD consortium, based in Toronto Canada at the Hospital for Sick Children and from the C4CMMRD

database, based in Villejuif, France at Gustave Roussy Cancer Campus. The IRRD consortium was established in 2007 to assess individuals suspected of having syndromes with replication repair deficiencies, including a large cohort of CMMRD patients (n=110), originating from 45 countries. Updates on surveillance and health status are obtained at regular intervals. The European Care for CMMRD (C4CMMRD) consortium was established in 2013. One of its objectives was to create a database dedicated to CMMRD patients. This database contains retrospective and prospective data, from 15 countries, with approximately 100 patients with confirmed CMMRD diagnosis. It collaborates with other national databases in European countries to address specific issues about CMMRD.

Based on literature review and expertise from the panel, draft clinical criteria were established by the working group at an International Replication Repair Deficiency workshop held in Toronto on Oct 15-16, 2017. The draft clinical criteria from the working group were presented to all workshop attendees, made up of 60 experts from 18 countries for input and consensus vote. The agreed upon criteria were further evaluated through an in-depth survey sent to the 13 members of the working group and completed by 8 members of the group by April 23, 2018. If consensus of 80% was reached, the criterion was adopted. A follow-up conference call with all members was arranged to discuss criteria that did not reach 80% agreement for review to eliminate or re-vote. Hypothetical cases as well as families with typical and atypical presentations of CMMRD from the IRRD consortium were reviewed to test the draft criteria. A second survey to finalize data was completed by February 28, 2019 by all members of the working group. The focus of this working group was development of criteria to determine who has a CMMRD diagnosis, thereby necessitating surveillance. We did not determine how to definitively rule out a diagnosis of CMMRD, which may be explored in a future document.

Results:

CMMRD diagnostic criteria

The expert panel established 6 diagnostic criteria outlined in Table 2; four criteria with strong evidence of CMMRD diagnosis (i.e. definitive diagnosis) and two criteria with moderate evidence of CMMRD (i.e. likely diagnosis). Both definitive and likely diagnostic criteria would

warrant CMMRD surveillance per published guidelines. The working group used 3 components for diagnosis including; (a) MMR germline results, (b) ancillary testing, and (c) clinical manifestations. Multi-gene panel testing is recommended for all suspected individuals to investigate overlapping conditions which can mimic this syndrome. Ancillary testing and clinical manifestation, including a new definition of CMMRD hallmark tumors, were explored in detail.

It is the recommendation of this working group that the diagnostic criteria established be reassessed as data emerges and as the criteria are applied to newly identified families suspected of CMMRD. The committee did not establish criteria to eliminate the suspicion of CMMRD and recognizes there may be families that meet a level of suspicion for this condition, but do not meet the strict evidence to confirm diagnosis. Clinical judgment should be used in all cases.

The internationally agreed upon diagnostic criteria, using a combination of clinical manifestations, genetic testing and ancillary molecular assays, are outlined in Table 2.

Rationale for Criteria

A. Mismatch repair genes germline testing

The American College of Medical Genetics (ACMG) outlined 5 variant classifications for germline analysis of Mendelian disorders; pathogenic (P), likely pathogenic (LP), variants of unknown significance (VUS), likely benign (LB) and benign (B) variants.²¹ Actionable variants include P/LP, while a VUS should not be used for clinical decision-making, and LB/B variants can be assumed not to cause phenotype.

Non definitive mismatch repair (MMR) germline results have been described in multiple CMMRD patients, including cases with biallelic VUS or combinations of VUS and P/LP variants.^{4, 22, 23} This suggests the VUS was misclassified and is pathogenic or that the VUS may not be clinically actionable as a monoallelic variant, it may sufficiently impact protein function when in combination with a second VUS or P/LP variant. One example of the complexity of molecular VUS results were reported by Taebner et al (2018) who found both homozygous *MSH2* and *MSH6* variants in a 13 month old with desmoplastic medulloblastoma and striking skin pigmentation using ancillary assays.^{24, 25} It was concluded that CMMRD was caused by the *MSH6* homozygous VUS.²⁶

A review of the literature outlined suspected CMMRD families in the absence of definitive biallelic pathogenic MMR variants, or based solely on clinical phenotype and ancillary testing.^{3, 4, 23, 27} A study by Bakry et al (2014) reported MMR germline results for 12 CMMRD families; 67% (n=8) had P/LP biallelic MMR variants, 17% (n=2) had biallelic VUS, 8% (n=1) had monoallelic PV, and 8% (n=1) had no mutation identified.²⁷ Similar distribution was seen in 38 families from the C4CMMRD consortium with 74% (n=28) P/LP biallelic MMR variants, 18% (n=7) biallelic VUS, and 8% (n=13) monoallelic PV and no monoallelic VUS.²⁸

This highlights the need to define criteria for ancillary assays that will diagnose CMMRD in the absence of confirmatory MMR germline testing mutation analysis.

B. Ancillary Testing

Ancillary testing, including assays showing microsatellite instability (MSI) in constitutional tissue, functional assays showing loss of MMR activity and MMR immunohistochemistry (IHC) showing loss of MMR protein expression may be used to support a diagnosis of CMMRD.

IHC analysis in non-neoplastic tissue (i.e. skin biopsy or adjacent normal tissue to a cancer) is the most widely available and least expensive test to assess for absence of MMR protein, however, interpretation is subjective and obtaining normal tissue can be invasive.²⁷ Pathologists must be aware of the suspected diagnosis as the positive stain which serves as an internal control in LS patients is often absent in all tissues of individuals with CMMRD. In addition, pathogenic missense variants, frequent in CMMRD, can lead to false normal results, where the protein is present and staining, but is non-functional, thereby leading to a false negative result.²⁹ Despite limitations, IHC of non-neoplastic tissue has higher than 90% sensitivity and specificity in experienced pathology laboratories and can be a useful tool in the diagnosis of CMMRD.

Functional assays have been developed, although widespread access at commercial laboratories may be limited. While conventional MSI testing using an NCI panel of mono- and dinucleotide repeat markers were validated for adult-onset Lynch syndrome cancers,³⁰ it is not sensitive at detecting MSI in CMMRD. A method to detect MSI in non-neoplastic tissue termed germline MSI (gMSI) has been developed, and this assay relies on the analysis of ‘stutter’ peaks typically

associated with microsatellite PCR products.²⁴ Its main limitation is that it uses dinucleotid microsatellites and, therefore, is insensitive to an *MSH6* deficiency. It is routinely used for diagnosis purposes in some European countries and allows for rapid results.

Another functional assays includes a combination of tolerance to methylating agents (a characteristic of MMR deficient cells) and MSI from lymphoblastoid cell lines called ex-vivoMSI (evMSI).²⁵ A presumptive diagnosis of CMMRD using this assay requires both tolerance to methylating agents and MSI to be concordant. A diagnosis of CMMRD can be ruled out if both tests show normal activity. The method is 100% sensitive and 100% specific. In cases of discrepancy, no conclusion can be made and another test should be considered. A limitation of this test is the timing necessary for immortalization and culture cells for evMSI (at least 120 days). This assay is available in routine diagnosis in France in an accredited laboratory and since January 2015, 77 patients have been tested prospectively with the two functional assays and 15 had both abnormal results consistent with a diagnosis of CMMRD (personal communication of M Muleris and C Colas). These assays are not easily accessible clinically in North America.

An *in vitro* repair assay was developed to quantify MMR activity from patient-derived lymphoblastoid cell lines.³¹ Testing a series of patients with CMMRD, Lynch syndrome, NF1, Li-Fraumeni, and polymerase proofreading-associated polyposis syndrome, the assay demonstrated high specificity and sensitivity. The assay requires live cell cultures as mismatch repair proteins are maximally produced during cell division, which limits its scalability. In addition, the assay is a complementation assay which allows for the determination of the defective protein complex (MSH2-MSH6 or MLH1-PMS2). This assay is not yet established as a clinically approved test, but is available through the IRRD consortium.

More recently next-generation sequencing (NGS)-based methods have proven to be very sensitive and specific at diagnosing CMMRD as they can detect low level of MSI from constitutional tissue.^{32, 33} NGS-based methods for MSI detection have the advantage of being easily scalable, cost effective.

Pediatric tumors showing hypermutation (>10 mutations/Mb) and MMR deficient signatures (single base substitution signatures 6, 15, 20, 26 and 44, doublet base substitution 7 and 10, small insertion and deletion signatures 1 and 2), may raise the suspicion of CMMRD.³⁴ Cerebral

tumors related to CMMRD are also associated with concurrent somatic mutations in the proofreading domain of the polymerases *POLE/POLD1*, which leads to ultrahypermutation phenotypes with mutation loads in excess of 100 mutations/Mb and specific *POLE/POLD1* signatures (single base substitution signature 14 and 20).³⁵ It is also noted dMMR in a malignancy is not sufficient to confirm a diagnosis of CMMRD as these features can also be present in tumors from patients with Lynch syndrome and even sporadic cancers that have acquired biallelic hits in a MMR gene.^{35, 36} As IHC deficiency in tumours, TMB and signature cannot clearly differentiate between LS, somatic biallelic MMR and CMMRD, the working group does not support using these as an ancillary test to confirm CMMRD at this time. It can be used to support a diagnosis once outlined diagnostic criteria are met.

The working group recommends using ancillary testing in challenging cases, and should be used to interpret atypical presentation of CMMRD or inconclusive germline molecular results within the clinical context. If a discrepancy occurs among tests, additional ancillary tests, preferably by orthogonal methods, should be performed to reach a more conclusive decision. Consideration may be given to implement tests that are already published with high sensitivity and specificity in accredited (e.g. CAP inspected) laboratories authorised to give a clinically usable report. The working group gives a framework of current ancillary tests, however a definitive list was not incorporated as new functional assays may be developed over time. A summary of the current tests outlining pros and cons is provided in Table 3.

C. Clinical Presentation

Since genetic and molecular criteria may be insufficient in the diagnosis of CMMRD, the working group defined specific clinical parameters to add in the establishment of CMMRD diagnosis.

C.i) Cancer Type and Age of Presentation

In order to assess criterion for clinical diagnosis, the working group reviewed most common primary malignancy sites, as well as typical age of first presentation. Atypical cancer site and range of age of diagnosis was also assessed to determine the full spectrum of CMMRD.

Literature review of several larger published CMMRD cohorts that outline primary cancer site, as well as unpublished data from the IRRD consortium and the C4CCMMRD database are summarized in Table 4. It reveals that 86-100% of individuals with CMMRD have reported a cancer diagnosis.^{3, 4, 37, 38} This may be a result of ascertainment bias, because cancer is the most common criterion leading to the diagnosis of CMMRD in index cases. Penetrance may also be dependent on timing of the publication as new cancers are likely to develop over time. In fact, one publication reported that at the time of recruitment, 19 of 23 (82.6%) individuals had a history of cancer, but by the time of publication, 22 individuals were affected (95.6%).²⁷

Wimmer et al (2014) outlined the most common CMMRD tumors and age of first diagnosis to include; WHO grade III/IV glioma <25 yrs, NHL of T-cell lineage < 18 yrs, GI adenocarcinoma <25 yrs, or GI adenomatous polyposis < 18 yrs.¹ These represent 60-84% of first diagnosis in CMMRD as outlined in Table 4.^{3, 4, 37, 38} We acknowledge there may be an overlap of cohorts within the two large consortiums; the IRRD consortium and C4CCMMRD database, therefore, we used recent, unpublished information from these two databases only to examine the common cancers outlined above. By expanding the criteria to all subtypes of brain, cerebral and hematological malignancies, an additional 18.75% (18/96) of the IRRD cases and 34% (31/90) from the C4CCMMRD database would be included as primary malignancies. The contribution of GI cancers as the primary diagnosis was also assessed, and accounted for 15% (15/96) of IRRD cases and 18% (16/90) of C4CCMMRD cases, most often reported as colorectum or small bowel cancer. Other studies have reported adenomatous polyposis as the first diagnosis, often diagnosed under age 18.^{4, 27, 39} Based on expertise and literature review, this working group defined new “hallmark tumors” in CMMRD as: (a) glioma or CNS embryonal tumors <25 yrs, (b) hematological cancer (excluding Hodgkin’s lymphoma) < 18 yrs, (c) gastrointestinal adenocarcinoma <25 yrs, and (d) gastrointestinal adenomatous polyposis of more than 10 adenomas < 18 yrs (after ruling out polyposis conditions). Using this definition of hallmark cancers, it would account for 76-96% of primary diagnoses reported in Table 4.

While the majority of CMMRD cases present with a hallmark tumor, there is a broader spectrum of less common primary and metachronous malignancies. These include embryonal tumours (e.g.

neuroblastoma, retinoblastoma, Wilms tumour), germ cell tumours (i.e. yolk sac tumour), sarcomas (e.g. osteosarcoma, dermatofibrosarcoma protuberans, rhabdomyosarcoma), ganglioneuroma, melanoma, urinary tract (bladder, kidney, ureter), prostate, breast, gynecological (endometrial, ovarian), and other GI (ampullary, gastric) cancers.^{1, 3, 4, 23, 27, 38-42}

Older age of onset of primary cancer has been noted in CMMRD and data for the larger studies are summarized in Table 4. Using unpublished data from the two largest consortiums (IRRD and C4CMMRD, respectively), 11-15.6% of cases presented at age 18 or older, and 1-7% at age 25 or older. The oldest age at first malignancy reported in CMMRD was rectal cancer at age 40 in an individual with *in trans* P/LP *PMS2* variants (c.2007-?_2589+?del (del 12-15)/ c.614A>C) who had confirmed loss of *PMS2* protein in tumor and non-neoplastic tissue.⁴² There are also atypical cancer sites diagnosed at older ages as the first malignancy in 2 reported CMMRD cases who presented with breast cancer over age 25.^{43,40} Older ages of onset of first cancer appears to be more commonly associated with epithelial cancers, although older onset lymphoma (diagnosed at age 33) has also been reported.⁴⁴ Genotype-phenotype correlations may explain some of this older onset cases. For example Li et al. (2015) described a founder homozygous PV of *PMS2* in Inuit families displaying an attenuated phenotype with a delayed average age of onset of cancer associated to a residual *PMS2* expression.⁷ These clinically unusual cases highlight the spectrum of CMMRD presentation and the challenges with establishing a diagnosis based on clinical presentation alone.

C.ii) Benign manifestations

Individuals with CMMRD often have features that are found in NF1, including café-au-lait macules (CALM), neurofibromas and axillary freckling. Only a minority of individuals with CMMRD meet the NIH diagnostic criteria for NF1, although 18.5% (n=27/146) have more than one NF1 feature and between 62-95% have CALM, albeit not all reach the threshold of ≥ 6 CALM.^{1, 4, 45}

Conversely, CALM are common in the general population, often present at childbirth but can continue to grow and develop with age. The incidence, varying with age and race/ethnicity, is reported in 0.3% to 1.8% of White individuals between birth to childhood years, and in 18% to

27% within that age range in individuals who identify as Black.⁴⁶ In addition to CMMRD and NF1, CALM can be associated with other hereditary conditions including Legius syndrome (*SPRED1* gene), Noonan syndrome (occurring with multiple lentiginos), other RASopathies, and McCune–Albright syndrome (often mosaic and occurring with skeletal anomalies). Hypochromic spots, not present in NF1, have been described in 16% (Lavoine et al) to 29% (unpublished C4CMMRD data) in CMMRD patients who have undergone a dermatological examination.⁴ In case of suspicion of CMMRD, a complete and careful skin examination must be performed and may provide elements in favor of the diagnosis.

Other clinical features reported in CMMRD patients include agenesis of the corpus callosum, grey matter heterotopia⁴⁷, venous anomalies⁴⁸, multiple pilomatrixoma⁴⁹, paediatric systemic lupus erythematosus⁵⁰, intracranial tuber-like lesions and renal angiomyolipoma.⁵¹ Decreased IgA and/or IgG2/4 levels¹ have also been reported, however, this was not substantiated in a recent study that could not consistently identify clinical or routine immunological laboratory parameters suggestive of primary immunodeficiency in 15 unrelated CMMRD patients.⁵² These abnormalities have no clinical impacts in terms of immunodeficiency for the vast majority of patients.⁵² While these manifestations have been observed, they are not routinely investigated at the time of CMMRD diagnosis. However, examinations of these benign manifestations can be of value, especially when diagnosis is doubtful, as they may provide arguments in favor of the diagnosis.

CMMRD is a legitimate differential diagnosis in children suspected to have sporadic NF1 in whom no *NF1* or *SPRED1* mutation is identified, as testing for CMMRD in these children would allow for cancer surveillance before their first malignancy. However, given that counseling and testing for CMMRD in these children is associated with potential harm and the estimated prevalence of CMMRD in these children is only 0.39%, the C4CMMRD consortium (Suerink et al 2018) developed criteria to preselect those children where CMMRD is more likely. The testing criteria outlines that an individual be tested if they fulfill the prerequisites (a) the presence of at least one diagnostic NF1 feature including at least two CALMs (b) absence of *NF1* and *SPRED1* germline mutations, (c) absence of NF1 signs in both parents. In addition the individual should have one or more features indicative of CMMRD in the family including: parental

consanguinity, Lynch syndrome in one or both parents, siblings with NF1 features or childhood malignancy, LS-related cancer diagnosed before age 60 in first-degree or second-degree relative; or personally: atypical CALMs (irregular borders and/or pigmentation), hypopigmented skin patches, ≥ 1 pilomatricoma, agenesis of the corpus callosum, non-therapy-induced cavernoma or multiple developmental vascular abnormalities.¹⁶

D. Multi-gene panel testing to search for differential diagnoses

The differential diagnosis of CMMRD includes a wide range of other hereditary tumor conditions. Lynch syndrome and CMMRD are both caused by mismatch repair gene mutations, and while they phenotypically differ, atypical cases can mimic each other. There have been rare cases of LS with glioblastoma or GI cancers diagnosed in childhood or young adulthood, and CMMRD cases presenting with adult-onset cancer.⁵³⁻⁵⁵ Polyposis conditions can also mimic CMMRD including individuals with specific *POLE* variants (i.e. p.V411L or p.A456P) who present with childhood hypermutant brain and colorectal cancers.^{56, 57} Conversely, individuals with CMMRD have also presented with adenomatous polyposis (i.e. 10-100 adenomas) mimicking conditions such as Familial Adenomatous Polyposis (FAP or attenuated FAP) and *MUTYH*-Associated Polyposis (MAP). There is also a rare case presenting with ≥ 30 juvenile polyps typically associated with Juvenile Polyposis Syndrome (JPS).⁵⁸ Early-onset malignancies can also overlap with Li-Fraumeni syndrome, caused by germline *TP53* mutations, which increases the risk for hematological, sarcoma, brain and GI cancers presenting at early ages.⁵⁹

There has also been overlap with conditions that have CALM, skin manifestation such as NF1 and Legius syndromes,^{45, 60} and tuberous sclerosis⁵¹. Parallel to MMR analysis, other syndromes should be searched through multi-gene panel testing encompassing hereditary colorectal and

polyposis conditions, childhood malignancy conditions (i.e. *TP53*), or syndromes based on skin findings (i.e. NF1, Legius, tuberous sclerosis) according to clinical presentation.

Other important diagnostic scenarios.

Full Siblings

If a proband meets diagnostic or likely diagnostic criteria, our expert panel recommends CMMRD surveillance for full siblings until CMMRD is ruled out by at least one of the following methods (Note: LS diagnosis may still be possible):

- From criteria 1, 2 or 5: if siblings did not inherit one of the familial variants as identified in the index case.
- If sibling(s) reaches age 30 years with no history of colorectal polyps or CMMRD related cancer
- If sibling has negative ancillary testing (when index case has proven positive ancillary test)

Consideration for *EPCAM*

To date, there is one known case of homozygous *EPCAM* 3'UTR deletions, unpublished and followed by the IRRD consortium. This 5-year old has colorectal polyposis, small bowel adenomas and 1 CALM. There has also been a 9-year old child reported to have compound heterozygous *MSH2* mutation and *EPCAM* 3'UTR deletion (*in trans*), with 70-80 colorectal adenomas, and two synchronous colorectal cancers.⁶¹ To date, there have been no reports of biallelic *EPCAM* variants with deletions extending into *MSH2*, and while rare, one might expect this to resemble the phenotype of biallelic *MSH2*.

At this time, there is not enough clinical information to determine the impact on biallelic *EPCAM* 3' UTR deletions on cancer risks and whether it is isolated to the GI tract, or if it extends to the other hallmark cancers of CMMRD. This may be also dependent on the involvement of the *MSH2* gene, as homozygous deletions that extend into *MSH2* would be

suspected to cause CMMRD. The working group recommend CMMRD surveillance for biallelic *EPCAM* 3' UTR deletions or individuals with *EPCAM/MSH2*, until further information becomes available.

Consideration for *MSH3*

There have been few reports of individuals with biallelic *MSH3* described as having adult onset adenomatous polyposis of the colon.⁶² Thyroid and small bowel adenomas, and astrocytoma have also been reported, all occurring in young adulthood.⁶² It is unclear whether individuals with biallelic *MSH3* require the same intensive screening as CMMRD patients, and at this time our expert panel concluded that insufficient evidence exists at this time to determine if cancer risk would be similar to CMMRD or FAP, and does not recommend CMMRD surveillance at this time.

As information continues to evolve on biallelic *EPCAM* and *MSH3*, as well as the expanding spectrum of CMMRD, the diagnostic criteria and surveillance recommendations should be assessed periodically.

Summary

This is the first consensus for diagnostic criteria for CMMRD which was achieved by a working group of international experts, including members of the IRRD and the C4CMMRD consortia who are involved in the management of most CMMRD individuals. The six diagnostic criteria described allows for clear surveillance recommendations and familial genetic counseling.

CMMRD is a condition with a variable clinical presentation encompassing a broad spectrum of cancers that may mimic other hereditary conditions. This working group defined a set of hallmark cancers, while recognizing there is variability in presentation of cancer site and age of onset. Benign manifestations are also broad and include features that overlap the phenotype of NF1, tuberous sclerosis, juvenile and adenomatous polyposis. Molecular testing is unable to conclusively identify biallelic pathogenic MMR gene variants in up to 40% of patients. The diagnostic criteria outlined is essential given the complexity of the syndrome.

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Tables and Figures

Figure 1: Wimmer et al (2014): Scoring system to determine germline testing eligibility for CMMRD¹

Indication for CMMRD testing in a cancer patient ≥ 3 points

Malignancies/premalignancies: one is mandatory; if more than one is present in the patient, add the points:

Carcinoma from the LS spectrum* at age <25 years	3 points
Multiple bowel adenomas at age <25 years and absence of APC/MUTYH mutation(s) or a single high-grade dysplasia adenoma at age <25 years	3 points
WHO grade III or IV glioma at age <25 years	2 points
NHL of T-cell lineage or sPNET at age <18 years	2 points
Any malignancy at age <18 years	1 point

Additional features: optional; if more than one of the following is present, add the points

Clinical sign of NF1 and/or ≥ 2 hyperpigmented and/or hypopigmented skin alterations $\varnothing > 1$ cm	2 points
Diagnosis of LS in a first-degree or second-degree relative	2 points
Carcinoma from LS spectrum* before the age of 60 in 1 st , 2 nd or 3 rd -degree relative	1 point
A sibling with carcinoma from the LS spectrum*, high-grade glioma, sPNET or NHL	2 points
A sibling with any type of childhood malignancy	1 point
Multiple pilomatricomas in the patient	2 points
One pilomatricoma in the patient	1 point
Agnesis of the corpus callosum or non-therapy-induced cavernoma in the patient	1 point
Consanguineous parents	1 point
Deficiency/reduced levels of IgG2/4 and/or IgA	1 point

*Colorectal, endometrial, small bowel, ureter, renal pelvis, biliary tract, stomach, bladder carcinoma. CMMRD, constitutional mismatch repair deficiency; LS, Lynch syndrome; NHL, non-Hodgkin's lymphomas; sPNET, supratentorial primitive neuroectodermal tumors

Table 1: Example of Surveillance protocol for patients with CMMRD

Examination	Start age	Frequency	Tumors	Comment
MRI brain	At diagnosis	Q 6 months	Brain tumors	Should not be replaced with WBMRI
WBMRI	6 years	Once a year	All tumors	Should not replace dedicated CNS imaging
CBC	1 year	Q 6 months	Leukemia	May be considered
Abdominal U/S	1 year	Q 6 months	Lymphoma	May be considered
Upper gastrointestinal endoscopy; VCE, ileocolonoscopy	4 to 6 years	Once a year	Gastrointestinal tumors	Upper and lower endoscopy, to increase once polyps are found
GYN exam, transvaginal U/S, pipelle curettage, urine cytology,	20 years	Once a year	Genitourinary cancers	As per LS guidelines

dipstick

Abbreviations: GYN, gynecologic; Q, every; U/S, ultrasound; VCE, visual capsule endoscopy.
(adapted from Tabori et al Clin Cancer Res (2017)¹⁷)

Table 2: CMMRD Diagnostic criteria

Criterion	Germline result* <i>PMS2, MSH6, MSH2, MLH1</i>	Positive Ancillary testing [#]	Clinical Phenotype	
Definitive Diagnosis (Strong evidence of CMMRD)	1	Biallelic pathogenic variants (P/P)*, confirmed <i>in trans</i> [^]	Not required unless unaffected >25 yrs, then one required [#]	Not required if under age 25 (if no malignancy over age 25, ancillary testing required)
	2	Biallelic P/LP or LP/LP* variants, confirmed <i>in trans</i> ^{^^}	One required [#] unless unaffected by hallmark cancer ^{>} , then 2 required	Hallmark CMMRD cancer diagnosis ^{>} or C4CMMRD criteria of 3 points ⁺ (then 2 ancillary tests required)
	3	Heterozygous P or LP variant (+/- VUS* or likely benign variants)	One required [#]	Hallmark CMMRD cancer diagnosis ^{>}
	4	No P or LP MMR variants (including VUS/VUS)** or No testing available (i.e. deceased proband)	Two required [#]	Hallmark CMMRD cancer diagnosis ^{>}
Likely Diagnosis (Moderate evidence of CMMRD)	5	Biallelic P/LP* or LP/LP variants confirmed <i>in trans</i> ^{^^}	Not required	C4CMMRD criteria of 3 points ⁺
	6 [^]	Heterozygous P or LP variant or no testing available (i.e. deceased proband)	Two required [#]	a. C4CMMRD criteria of 3 points ⁺ b. Individual < age 18 with NF1 features (i.e. no malignancy or polyposis history) c. Malignancy under age 30

* Biallelic—impacts same gene on both parental alleles (i.e. *PMS2/PMS2*), P—pathogenic (ACMG C5), LP—likely pathogenic (ACMG C4), VUS—(ACMG C3). Multi-gene panel testing is recommended to investigate overlapping conditions. Consider phenotype of individual to rule out overlapping syndromes. All families should be assessed in a specialized centre for diagnosis.

** Consanguinity further support a diagnosis of CMMRD due to a homozygous MMR gene mutation that is unidentifiable

[^] In trans variants can be proven by testing parents, offspring or other relatives. If unavailable to confirm variants *in trans*, individual should fulfil criterion #3

^{^^} If unavailable to confirm variants *in trans*, individual should fulfil criterion #6

[#] Ancillary testing is described in further detail above. Does not include tumor mutation burden and signature at this time. Functional testing should be published with proven high sensitivity and specificity performed in an accredited (e.g. CAP inspected) laboratory authorised to give a clinically usable report. If discrepancy occurs among tests, multiple ancillary tests should be used to reach more conclusive decision.

[>] Hallmark CMMRD cancer: Glioma or CNS embryonal tumors <25 yrs, Hematological cancer (excluding Hodgkin's lymphoma) < 18 yrs, GI adenocarcinoma <25 yrs, or >10 adenomatous GI polyps < 18 yrs (after ruling out polyposis conditions).

⁺ C4CMMRD criteria outlined in Figure 1

^ Individuals with two positive ancillary tests for CMMRD in the absence of the described phenotype, can be assessed on a case-by-case basis, but these are atypical CMMRD cases and additional assessment is required to determine surveillance.

Table 3: Examples of Ancillary tests available to assist in CMMRD diagnosis

Test	Pros	Cons	References
Germline MSI (gMSI)	<ul style="list-style-type: none"> • Rapid result • Specific for CMMRD 	<ul style="list-style-type: none"> • May be insensitive to MSH6 deficiency • Not widely available commercially outside Europe 	Ingham et al. (2013) Human mutation. 34(6):847-52.
Ex-vivo MSI (evMSI) + methylation tolerance	<ul style="list-style-type: none"> • High sensitivity and specificity 	<ul style="list-style-type: none"> • Discordant results between test may require additional ancillary testing • Time to develop lymphoblastic cell line • Not widely available commercially outside Europe 	Bodo et al (2015) Gastroenterology. 149(4):1017-29.e3
<i>in vitro</i> repair assay	<ul style="list-style-type: none"> • High sensitivity and specificity 	<ul style="list-style-type: none"> • Time to develop lymphoblastic cell line • Not widely available commercially (limited scalability) 	Shuen et al (2019) Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 37(6):461-70.
NGS detection low level MSI in tissue	<ul style="list-style-type: none"> • Sensitive and specific for CMMRD • Cost effective and scalable 	<ul style="list-style-type: none"> • Not widely commercially available 	Gallon et al (2019);Hum Mutat.40(5):649-55. Gonzalez-Acosta et al. (2020) J Med Gen. 57(4):269-273.
Immunohistochemistry of the 4 MMR genes.	<ul style="list-style-type: none"> • Easily accessible • Inexpensive • High specificity and sensitivity 	<ul style="list-style-type: none"> • False positives and negative can occur • Interpretation must be made with care and is operator dependent • Access to non-neoplastic tissue may be invasive 	

Table 4: Age and site of first malignancy as reported in large CMMRD series

Cancer site	References with cancer per site, median age in years (range)											
	IRRD consortium (unpublished) (n=96)		C4CMMRD consortium (unpublished) (n=90)		Lavoine et al ⁴ (n=31)		Baris et al ³ (n=14)		Durno et al ³⁷ (n=29)		Wimmer and Etzler ³⁸ (n=78)	
	n=	median (range)	n=	median (range)	n=	median (range)	n=	median (range)	n=	median (range)	n=	median (range)
Brain – WHO grade III/IV glioma	44	9.3 (2-27)	22	7.71 (0.1-18)	9	6 (3-19)	3	12 (2-13)	4	12 (6-19)	20	7 (2-19)
GI cancer	15	16.7 (8-40)	16	18.43 (7-33)	7	21 (19-33)	3	18 (14-20)	16	23 (5-18)	21	17 (9-35)
>10 GI adenomas	0		0		1	14	3	11 (9.5-16)	0		2	8.5 (6-8)
T-cell lymphoma	8	6.3 (3-27)	18	4.42 (0-15)	9	4 (1-6)	1	6	2	4 (2-6)	7	2.5 (0.4-10)
Brain – other*	9	9 (5-16)	18	6.65 (1-22)	1	5	2	5.5 (5-6)	1	7	8	7.5 (4-14)
Other hematological cancer[^]	9	8 (2-30)	13	5.34 (1.5-15)	1	6	0		0		13	4 (1-15)
Other cancer[#]	5	2.8 (1-29)	3	3.17 (0-11.15)	3	11 (3-21)	0		2	9.5 (4-15)	4	8.5 (1-35)
Unaffected	6		1	10	0		2		0		3	7 (6-10)
Wimmer et al¹ defined cancers⁺	64.5% (n=62)		62.2% (n=56)		83.9% (n=26)		71.4% (n=10)		72.4% (n=21)		60.2% (n=47)	
Hallmark cancers[~]	82.3% (n=79)		96% (n=87)		90.3% (n=28)		92.8% (n=13)		75.8% (n=22)		87.2% (n=63)	
Overall dx \geq18	15.6% (n=15)		11.1% (n=10)		32% (n=10)		14.3% (n=2)		37.9% (n=11)		14.1% (n=11)	
Overall dx \geq25	7.3% (n=7)		1.1% (n=1)		3.2% (n=1)		0		3.4% (n=1)		3.8% (n=3)	
Overall with malignancy	93.7% (n=90)		98.8% (n=89)		100% (n=31)		85.7% (n=12)		100% (n=29)		96.1% (n=75)	

* Medulloblastoma, primitive neuroectodermal tumor (PNET), supratentorial primitive neuroectodermal tumors (sPNET)

[^] B or T-cell ALL, AML, CML Follicular lymphoma, Burkitt Lymphoma, NHL unspecified

[#]: Breast, neuroblastoma, retinoblastoma, Wilms tumor, yolk sac tumor, ganglioneuroma, osteosarcoma, DFS protuberans, rhabdomyosarcoma, renal pelvis carcinoma, myofibromatosis

[†]: WHO grade III/IV glioma <25 yrs, NHL of T-cell lineage < 18 yrs, GI adenocarcinoma <25 yrs, or GI adenomatous polyposis < 18 yrs

[~] Glioma or CNS embryonal tumors <25 yrs, Hematological cancer (excluding Hodgkin's lymphoma) < 18 yrs, GI adenocarcinoma <25 yrs, or >10 adenomatous GI polyps < 18 yrs (after ruling out polyposis conditions).