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Review article

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The intersection of homologous recombination (HR) and mismatch repair (MMR) pathways in DNA repair-defective tumors

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Homologous recombination (HR) and mismatch repair (MMR) defects are driver mutational imprints and actionable biomarkers in DNA repair-defective tumors. Although usually thought as mutually exclusive pathways, recent preclinical and clinical research provide preliminary evidence of a functional crosslink and crosstalk between HRR and MMR. Shared core proteins are identified as key players in both pathways, broadening the concept of DNA repair mechanism exclusivity in specific tumor types. These observations may result in unexplored forms of synthetic lethality or hypermutable tumor phenotypes, potentially impacting the cancer risk management, and considerably expanding in the future the therapeutic window for DNA repair-defective tumors.

DNA damage and impaired DNA repair processes are the main endogenous sources of genomic and, in particular, chromosomal instability (CIN)[1]. The genes that encode components specifically involved in DNA repair pathways, genome and chromosome integrity are the main drivers of hereditary cancers[2]. Today, identifying driver mutational imprints of DNA repair that both predispose to cancer development and establish drug vulnerabilities is one of the main goals in cancer research[3].

Within the network of known genome maintenance pathways, the key role of homologous recombination (HR) repair (HRR) and mismatch repair (MMR) emerges strongly from the observation that individuals carrying germline deleterious variants in HRR or MMR genes show a remarkably elevated lifetime risk for the development of several cancer types[4,5]. The mutated gene, the prevalence, and the DNA repair pathways involved varied across the cancer histologies[6–8].

Importantly, DNA repair pathways have been usually thought as mutually exclusive, with implications for genetic screening strategies and treatment stratification[9]. Thus, breast, ovarian, pancreatic, and prostate hereditary cancers seem dominated mainly by HRR repair deficiency (HRD), and are historically recognized as HRD-cancers[10]; similarly, colorectal and endometrial hereditary cancers are primarily characterized by microsatellite instability (MSI), caused by defects in DNA MMR, resulting in a characteristic mutational footprint[10,11].

Although the potential interactions between HRR and MMR mechanisms remain widely unexplored, recent research provides preliminary evidence of a functional crosslink and crosstalk between different DNA repair deficiencies in small subsets of cancers, greatly increasing the repertoire of defects in these critical pathways[12]. These observations may result in unexplored forms of synthetic lethality or hypermutable tumor phenotypes, considerably expanding in the future the therapeutic window for DNA repair-defective tumors[13]. Tumors known as HRD-cancers, but showing predominant MMR deficiency (MMRd) signature, could present an increased mutation load, and could be potential candidates for the treatment with immune checkpoint inhibitors (ICIs); conversely, a perturbed HR system in classically MSI-affected tumors, may represent an actionable biomarker for the treatments with poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi). A deeper understanding of the signature lesions of DNA repair processes is a critical need, and future evidences in the clinical context could

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represent a novel starting point to expand the possibility to target DNA repair-defective tumors.

This review aims to outline the current scenario of tumors identified as HRD- or MMRd-associated and the underlying molecular mechanisms, exploring the evidence of the intersection or dichotomy of these two DNA damage repair pathways.

Homologous recombination and mismatch repair: critical drivers of hereditary cancers

Genomic instability is a hallmark of cancer[14]. Cellular exposure to environmental or endogenous stresses can generate various types of DNA damage. Specific DNA damage response pathways are activated by cells in response to DNA damage. If unrepaired, the altered genetic information leads to the acquisition of specific mutations, which may predispose to cancer onset[15]. At least eight distinct DNA repair pathways can be activated to repair damaged DNA, including HR, MMR, base-excision repair (BER), nucleotide-excision repair (NER), non-homologous end joining (NHEJ), translesion synthesis (TLS), the Fanconi anemia (FA) and the O6-methylguanine DNA methyltransferase (MGMT) pathways[15].

The critical role of HR and MMR in DNA damage response (DDR) and genome maintenance was widely determined. Notably, a large fraction of known drivers of hereditary cancers are genes involved in these two DNA repair pathways[16] (Table 1).

Breast cancer susceptibility gene 1 (*BRCA1*) and breast cancer susceptibility gene 2 (*BRCA2*) are the main DNA repair genes linked to hereditary breast and ovarian cancer (HBOC)[17,18]. Currently, *BRCA1/2* pathogenic or likely pathogenic variants (PV/LPVs) are associated with an increased lifetime risk of other cancers, mainly prostate and pancreatic cancers[19], and the association between all these tumors and more moderate-penetrance genes in the HR pathway is continuously emerging[5,20]. Similar to the HRR, the MMR pathway plays an important role in hereditary cancers. Inactivating germline PVs in the MMR genes are the genetic background of Lynch syndrome (LS), an autosomal dominant disorder clinically also known as hereditary nonpolyposis colorectal cancer (HNPCC) as defined by Amsterdam and Bethesda criteria (Table 2)[21], associated with a significantly increased risk of several cancer types such as colorectal, but also endometrial, small bowel, gastric, ovarian, and ureteral cancers[22] (Fig. 1). Importantly, these DNA repair defects in either the HR or MMR pathways, are also current biomarkers for guiding the use of PARPi and ICIs, respectively[10,23].

However, deleterious variants in HRR or MMR genes only identified a low proportion of hereditary tumors[16]. Several inherited genetic drivers are still not fully understood. Understanding whether tumors associated with the HRD spectrum could arise in individuals with deficiencies in other DNA repair pathways, such as MMRd, and vice versa, would significantly increase opportunities for cancer risk management and therapeutic efforts.

HRR: role, crosstalk, and epistasis of key players

Among DNA damages, double-strand breaks (DSBs) represent the most damaging form of DNA lesions, resulting in deep and irreversible genomic wounds if not correctly healed[24]. The two main DSB repair pathways are the HR and the NHEJ, each of which is involved in different phases of the cell cycle with the first mainly involved during replication and the second throughout interphase[10]. The HRR is a highly conserved and accurate DNA repair pathway. In the absence of functional HRR, for example, when either *BRCA1* or *BRCA2* are defective, the preferential use of error-prone systems to repair DSBs leads to an increased burden of genomic alterations[25]. Deficiency in the HRR pathway is known as HRD, while tumors that are not HRD are termed homologous recombination proficient (HRP)[25]. The best-characterized HRR genes are certainly *BRCA1* and *BRCA2*: germline and somatic PV/LPVs, as well as epigenetic modifications in *BRCA1* and *BRCA2*, have been strongly associated with an HRD phenotype[26]. However, beyond *BRCA*, deleterious variants in HR-related genes other than *BRCA1/2*, such as Ataxia Telangiectasia Mutated (*ATM*), Partner and Localizer of BRCA2 (*PALB2*), checkpoint kinase 2 (*CHEK2*), RAD51 Recombinase (*RAD51*), BRCA1 Interacting Helicase 1 (*BRIP1*) and BRCA1 Associated RING Domain 1 (*BARD1*) genes, also confer an HRD or "BRCAness" phenotype. Their role in the DNA repair downstream pathway is well-defined and takes place through the interaction with both *BRCA1* and *BRCA2* genes[27].

The presence of a *BRCA* PV/LPV directs therapeutic management with PARPi in patients with breast, ovarian, pancreatic, and prostate cancers, leading to novel models of mainstreaming cancer genetics[19,28].

The significant relationship among all proteins involved in DSB repair expands the possibility of a successful HRD-PARPi synthetic lethality. In fact, several approved PARPis are not restricted to *BRCA1/2*-mutated patients. In ovarian cancer patients with HRD-positive tumors, even in the absence of a *BRCA1/2* PVs, recent clinical trials showed a clinically meaningful benefit of adding PARPi maintenance therapy, alone or in combination with bevacizumab, following response to platinum-based chemotherapy (PAOLA-1 and PRIMA trials)[29,30]. Although genomic scar assays provide information on the magnitude of PARPi benefits depending on HRD status, the optimal HRD biomarkers in this population are debated. At the same time, whether PARPi treatment can be proposed for cancer patients with non-*BRCA* HRR PV/LPVs remains controversial, highlighting how, beyond *BRCA1/2* PVs, HRR multigene panel and HRD genomic instability tests are not interchangeable[27].

The complementary effect between the HRR-related, non-BRCA, proteins, and BRCA1/2, reinforces the need for an enhanced definition of HRD biomarkers of PARPi effectiveness.

MMR: a mutator phenotype that reshapes the tumor microenvironment

DNA MMR is a highly conserved mechanism that enables the recognition and repair of randomly incorporated errors during DNA replication, significantly enhancing genomic stability. The mispaired nucleotides are caused by polymerase misincorporation errors, recombination events between DNA double helix strands, as well as chemical or physical damages[31]. The genes codifying for the MMR proteins are named as the homologous counterpart of *E. coli* system; mutS homologs (MSH2, MSH3, MSH4, MSH5, MSH6), mutL homologs (MLH1, MLH3), and post-meiotic segregation increased (PMS1, PMS2)[32].

All the proteins function as heterodimers allowing firstly the recognition and thereafter the repair of mispaired bases as well as small insertion/deletion. Several mechanisms can lead to MMR deficiency. The most common causes can be linked mainly to acquired somatic MMR mutations leading to gene function inactivation, along with *MLH1* gene silencing due to hypermethylation of the *MLH1* promoter region[33,34]. Deficiency can also occur due to germline mutations in the MMR genes. The germline mutations in the MMR genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*, or deletion at the 3' end of the *EPCAM* gene, which result in hypermethylation of the *MSH2* promoter[35] are the most commonly known cause of hereditary colorectal cancer (CRC); such mutations lead to the development of LS[36]. However, recently, *MLH1* hypermethylation has been reported in rare cases of patients affected by LS, although it is often linked to sporadic CRCs[37]. In particular, simultaneous loss of *MLH1* and *PMS2* expression is the most common pattern of LS based on *MLH1* germline mutations, followed by an *MSH2*/*MSH6* loss due to *MSH2* germline mutations[38]. The natural consequence of the presence of MMRd is MSI. Microsatellites are short tandem repeat DNA sequences of one to tetra base pairs distributed both in coding and non-coding regions of the human genome[39]. This instability arises as a consequence of the repetitive structure of microsatellites, which are particularly susceptible to replication errors normally repaired by the MMR system. However, recent findings have revealed that important mutational events are often a consequence of genomic destabilization and not only occur during replication, even in the presence of MMRd. This phenomenon is associated with the repair of DSBs occurring during replication stress through the microhomology-mediated end-joining (MMEJ) mechanism, mediated by enzymes such as DNA polymerase θ and PARP, as reported by Matsuno et al.[40]. Moreover, recent studies have raised several doubts about

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Table 1 | The lifetime cancer risk for the individuals carriers of pathogenic variants in high, moderate, or low penetrance genes involved in HRR or MMR pathways

DDR pathways	Gene(s)	Gene-related cancer-predisposing syndrome	MOI	Gynecological cancer risk		GI cancer risk		Other cancer risk (%)
				BC (%)	OC (%)	CRC (%)	PaC (%)	
High penetrance genes								
HRR	*BRCA1*	Hereditary Breast and Ovarian Cancer syndrome (HBOC); Fanconi anemia (FA)	AD/AR	>60 (F), 0.2–1.2 (M)	39–58	–	≤5	PrC (7–26) Melanoma, Endometrium, GC
HRR	*BRCA2*			>60 (F), 1.8–7.1 (M)	13–29	–	5–10	PrC (19–61) Melanoma, Endometrium, GC
Moderate and low penetrance genes								
HRR	*PALB2 (or FANCN)*	PALB2/BRIP1-associated hereditary cancer syndrome; Fanconi anemia (FA)	AD/AR	40–60	3–5	–	5–10	N/A
HRR	*BRIP1 (or FANCJ)*			–	5–15	N/A	N/A	N/A
HRR	*ATM*	ATM-associated hereditary cancer syndrome; Ataxia–telangiectasia (A–T)	AD/AR	20–40	2–3	5–10	5–10	PrC (30) B-cell lymphoma, Leukemia
HRR	*CHEK2*	CHEK2-associated hereditary cancer syndrome	AD	20–40	N/A	15	N/A	PrC
HRR	*BARD1*	BARD1-associated hereditary cancer syndrome	AD	20–40	N/A	N/A	N/A	N/A
HRR	*RAD51C*	RAD51C/D-associated hereditary cancer syndrome; Fanconi Anemia (FA)	AD/AR	20–40	10–15	N/A	N/A	N/A
HRR	*RAD51D*			20–40	10–20	N/A	N/A	N/A
MMR	*MLH1, PMS2, MSH2, MSH6*	Lynch syndrome (HNPCC); Constitutional Mismatch Repair Deficiency (CMMRD)	AD/AR	<15	MLH1 4–20 MSH2 8–38 MSH6 ≤ 1–13 PMS2 1.3–3	10–74	<5–10 (PMS2 excluded)	Endometrial (40–60) GC, Brain, Lymphoma, Leukemia
Lifetime risk of developing cancer in the general population								
–				13 (F)	1.1	4.1	1.7	PrC (12.9) Endometrial (3.1) Melanoma (2.2) GC (0.8) Thyroid (1.2) Kidney (1.8)

MOI mode of inheritance, *AD* autosomal dominant, *AR* autosomal recessive, *F* female, *M* male, *GI* gastrointestinal, *BC* breast cancer, *OC* ovarian cancer, *CRC* colorectal cancer, *DDR* DNA damage response, *PaC* pancreatic cancer, *GC* gastric cancer, *PrC* prostate cancer.
Modified by NCCN 2.2024.

Table 2 | Amsterdam I/II criteria and revised Bethesda guidelines for the selection of individuals with a higher risk of Lynch Syndrome

Amsterdam I criteria
There should be at least 3 relatives with colorectal cancer (CRC), and all the following criteria should be present:
- a first-degree relative of the other 2; - cancer involving at 2 successive generations; - at least one CRC diagnosed before the age of 50 years; - familial adenomatous polyposis must be excluded
Amsterdam II criteria
Three or more relatives with histologically verified Lynch sindrome-related tumors (colorectal, endometrial, small bowel, ureter or renal pelvis) of which:
- a relative should be a first-degree relative of the other two; - cancer involving at 2 successive generations; - one or more cancers must be diagnosed before the age of 50 years; - familial adenomatous polyposis should be excluded.
Revised Bethesda Guidelines
- Colorectal cancer diagnosed in a patient younger than 50 years; - Presence of synchronous or metachronous colorectal or other LS-associated tumors, regardless of age; - Colorectal cancer with MSI histology diagnosed in patients younger than 60 years; - Colorectal cancer diagnosed in a patient with one or more first-degree relatives with LS-related cancer, with one of the tumors diagnosed under age 50 years; - Colorectal cancer diagnosed in a patient with two or more first- or second-degree relatives with LS-related cancers regardless of age.

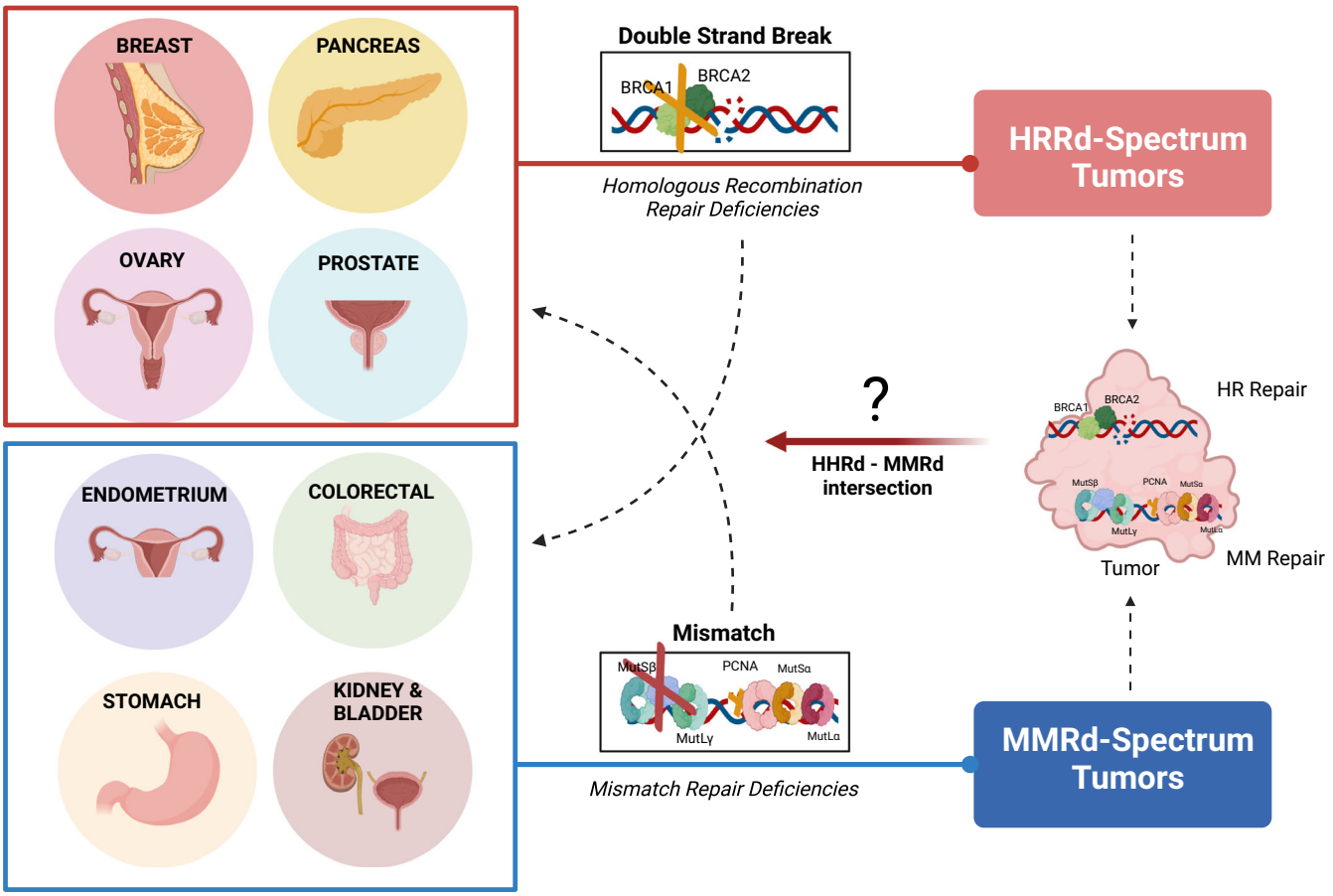

Fig. 1 | HRD- and MMRd-associated tumors. Classically, breast, ovarian, pancreatic, and prostate hereditary cancers are known as HRD tumors; while, colorectal and endometrium, but also additional cancers, including cancers of the stomach and renal pelvis, are classified as MMRd-associated tumors. Determination of MSI/MMRd as well as HRD status in different cancer types, beyond their characteristic mutational footprints, may improve genetic screening strategies and treatment stratification (Created with BioRender.com).

our understanding of immunogenicity in MMRd tumors. Indeed, the fact that MMRd tumors are typically associated with a rich immune microenvironment, with high infiltration of T lymphocytes and a consequent higher host anti-tumor response, represented the soil for the important benefits showed to immune checkpoint blockade, irrespective of the organ site of tumor origin[41]. For this reason, in the 2017, the US Food and Drug Administration (FDA) authorized the use of the PD-1 inhibitor pembrolizumab for the treatment of patients with solid unresectable or

metastatic tumors expressing MMRd/MSI, with progressive disease after prior treatment and without additional therapeutic options. The same treatment was approved for MMRd or MSI progressive CRC following fluoropyrimidine, oxaliplatin, and irinotecan[42,43]. Importantly, this is the FDA's first tissue/site-agnostic approval. In the same year, another anti-PD-1 drug, nivolumab, had obtained the FDA accelerated approval for the treatment of metastatic MMRd or MSI CRC with disease progression after standard chemotherapy[36].

Interestingly, while it was expected that the presence of MSI would lead to increased production of a larger reservoir of novel frameshift peptides, the development of neoantigens, and consequently, a stronger immune response, recently the results of preclinical studies have suggested a discrepancy regarding this simplistic model. Indeed, it has been found that, contrary to expectations, the majority of antigens presented in MMRd CRCs are not mutated[44], and antigens derived from mutated MHC-I-associated peptides are lost after the growth of such tumors in immunocompetent mice[45]. These findings revolutionize the neoantigen hypothesis as the sole explanation for the efficacy of ICIs in these tumors.

HRD and MMR/MSI testing in the clinic: still stuck at temporary snapshot?

HRD testing

The remarkable percentage of patients showing a BRCAness phenotype has opened numerous questions regarding new strategies and potential useful biomarkers for a proper patient selection in this specific setting. While the involvement and testing of *BRCA1/2* genes have been deeply clarified as a predictor of sensitivity to PARPi, insufficient evidence supports the testing recommendation for other HRR-related genes, including *ATM, ATR, PALB2, RAD51, RAD51B, RAD51C, RAD51D, BARD1, BRIP1*[27]. To date, several multigene panels, spanning from a small to a wider number of genes, have been exploited to identify PV/LPVs as well as large rearrangements affecting HRR-related genes. Consequently, depending on the available next-generation sequencing (NGS) platforms and size targets, different sequencing pipelines are thus needed, from amplicon-based to hybrid-capture-based target enrichment approaches. The former relies on different primer pool mixes for the selective amplification of DNA/RNA targets through multiple PCR steps. The latter, conversely, uses specifically designed small nucleic acid probes enabling the proper selection of common/unknown as well as larger regions of interest[46,47].

In the recent years, a growing interest has been particularly focused on the genomic instability as a direct effect of such genetic alterations. This level of genomic instability has brought many advantages in terms of tailored treatment opportunities as remarkably highlighted in high-grade serous ovarian cancer (HGSOC)[48]. In this context, to properly address patients likely to benefit from the administration of PARPis, the FDA has approved several tests among which the two spread and known worldwide are the NGS-based companions Myriad myChoice® CDx and FoundationOne CDx. Both testing strategies enable the identification of alteration at the genetic and genomic levels[49]. The Myriad myChoice® CDx allows the identification of single nucleotide variant (SNV)/indels/large rearrangements in several HRR-related genes along with the evaluation of the Genomic Instability Score (GIS) as a measure of loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST). A score greater than 42 is suggestive of HRR deficiency and this value has been clinically used to further target patients likely to benefit from PARPi administration. Moreover, the reliability of this parameter has been extensively adopted in several clinical trials, such as PRIMA and PAOLA-1[50]. The FoundationOne CDx (F1CDx) is a hybrid-capture NGS-based comprehensive genomic profiling (CGP) that allows the identification of multiple genetic alterations in 324 cancer genes along with tumor mutational burden (TMB) and MSI[51]. Furthermore, this companion testing allows the identification of the percentage of neoplastic tissue specimens showing genomic LOH. The percentage cut-off used has been 14% and 16% in the ARIEL 2 and ARIEL 3 randomized clinical trials, respectively[52]. Most recently, the measurement of RAD51 foci accumulation through immunohistochemistry (IHC) and/or immunofluorescence (IF), is gaining attraction as a novel approach to unraveling HRD[27,53].

In this scenario, several technical and clinical issues for the current testing and assays exist. Although our ability to stratify patients with HRD-related tumors improved the treatment selection, the different approaches that recognize the causes of HRD, such as the HRR deleterious variants, the functional alteration of HRR activity itself, such as the RAD51 foci assay, or the HRD consequences, through the genomic scar assays, are not interchangeable testing and require a better optimization in the clinic[27].

MMRd/MSI testing

Defective DNA MMR results, generally, in MSI in tumor tissue. For this reason, MSI is recognized as a hallmark of LS, and up to 30% of several cancer types[54].

The current tumor testing provides the possibility to identify LS by direct and indirect methods. The standard diagnostic procedure recommended by the National Cancer Institute (NCI) involves analyses of tumor and normal tissues using five microsatellite markers (Bethesda panel), including two for mononucleotide repeats (BAT-26 and BAT-25) and three for dinucleotide repeats (D2S123, D5S346, and D17S250)[33]. The ESMO recommendations on MSI testing for immunotherapy in cancer recommended the Bethesda panel, or an alternative panel with five poly-A mononucleotide repeats (BAT-25, BAT-26, NR-21, NR-24, NR-27), characterized by higher sensitivity and specificity[55]. MSI was defined as loss of stability in ≥ 2 out of the five microsatellite markers. As suggested in the revised Bethesda guidelines for CRC[33], and endorsed by the ESMO recommendations[55], the terms MSI-high and MSI-low should be overcome, and MSI-low grouped with microsatellite stable (MSS) tumors.

The IHC can indicate the presence or absence of a functional MMR system, and thus indirectly the presence of MSI. It allows to identification of the defective protein and then leads to the mutational analysis of the relevant gene[56]. The so-called universal tumor screening, followed by constitutional testing, estimated 3% of consecutive, unselected, colorectal and endometrial cancer patients, and 10–15% of tumors with MMRd, associated with LS[54,57].

However, one relevant observation should be made on MSI and MMR protein expression in tumor tissue of individuals with constitutional defects of MMR. Although immunohistochemical analysis showed the absence of one or more MMR proteins, the MSI frequency in the same tumor tissue varied according to the tumor type: 80–100% for the primary tumors of colon, stomach, ureter, and ovary; 50% for endometrium, bladder, and kidney tumors, and 35% for breast cancer[54]. At the same time, LS cancer patients with MSI tumors often showed tumors other than colorectal and endometrium, or canonical LS-spectrum tumors, such as prostate cancer, melanoma, soft tissue sarcoma, and mesothelioma[54].

Although IHC and pentaplex PCR remain the gold standard procedures, recent efforts have been made to develop NGS-based diagnosis tools, including the FDA-approved Memorial Sloan Kettering Cancer Center's (MSK)-Integrated Mutation Profiling of Actionable Cancer Targets (IMPACT) MSISensor algorithm. This technology showed that NGS improves recognition of patients with MSI in pan-cancer by comparing sequencing reads around microsatellite regions in the tumor and paired normal samples, and reporting the percentage of unstable loci as a cumulative score in the tumor[58,59].

Irrespective of the diagnostic method used, the diagnosis of LS ultimately requires constitutional genetic testing to identify the deleterious variants in the MMR genes. Constitutional sequencing-based variant detection is coming to a paradigm shift toward universal germline genetic testing[60]. Recent studies in unselected population for high-risk features showed 3% and 6% of LS among colorectal and endometrium cancers, respectively, investigated using hereditary multigene cancer panel testing[54]. Certainly, the possibility to detect all mutation carriers is appealing, but the frequent occurrence of variants of uncertain significance (VUS), or deleterious variants of uncertain clinical interpretation, makes the topic an ongoing debate.

In the future, novel minimally invasive options, such as the liquid biopsy[61] or the emerging "liquidomics"[62], could represent a dynamic and sensitive approach to simultaneously screening MMR and HRR-associated alterations, and to expand genetic and genomic knowledge on these tumors.

MMRd signature in the HRD-spectrum tumors: broadening the concept of DNA repair exclusivity

The evaluation of MMR and HRR status is essential in the clinical decision-making process to tailor both the diagnostic and therapeutic approach in the sporadic setting of MMRd and HRD tumors. Unsurprisingly, the inactivation of MMR genes in the germline leads to an increased susceptibility and earlier onset of various cancer types, a direct consequence of heightened mutation rates. The occurrence of inherited heterozygous mutations in the MMR genes is considered to be the hallmark of LS, implicated in the familial clustering of colorectal and endometrial cancers while also being associated with an elevated risk of other cancers, including but not limited to stomach, ovaries, prostate, and bladder[63]. Alternatively, biallelic germline mutations of one of the four MMR genes result in a distinct phenotypically and extremely aggressive cancer predisposition syndrome defined as Constitutional Mismatch Repair Deficiency (CMMRD), commonly predisposing to brain, gastrointestinal, and hematopoietic malignancies[64].

Although the majority of causal variants impact the core MMR genes, such pathogenic events are only detectable in a fraction of familial cancer cases[65]. Intriguingly, in around half of hereditary MMRd CRCs, a genetic cause cannot be identified, leading to a phenomenon known as "Lynch-like syndrome"[66]. One possible explanation for this limitation is that familial cancer risk may be attributed to conditions beyond LS, potentially involving epigenetic or somatic changes of modifier genes unrelated to MMR, such as *APC*, BER genes like *MUTYH* and *NTHL1*, or replicative DNA polymerases such as *POLE* or *POLD1*[67]. Recent discoveries have shown that biallelic somatic mutations in MMR genes are possible and can account for up to 50% of unexplained MMRd tumors. Additionally, although rare, somatic mosaicism has been observed, contributing to the complexity of understanding the genetic basis of MMRd tumors[68]. It could also be plausible that these individuals do have LS, but the sensitivity of current genetic testing technologies may be insufficient to detect the germline mutations in these cases. There are documented rare heritable causes of LS, such as constitutional *MLH1* hypermethylation and complex rearrangements of MMR genes, which may currently escape detection by clinically available genetic testing technologies[69].

The knowledge-driven advancement of immunotherapy designed to address both inherited and sporadic MSI cancers seems to complement the knowledge-based development of PARPis intended for the treatment of both inherited and somatic DNA DSB-associated cancers linked to *BRCA1/2* deficiency[70]. Mutational signatures might offer a potential avenue to elucidate relevant biological and mechanistic insights. Deconvoluting the diversity of somatic mutations and tumor mutational burden into individual mutational signatures would provide a powerful tool for identifying processes generating somatic mutations in different cancer types[71]. In this context, there is a clear imperative for additional studies aimed at uncovering the causes of unexplained familial tumor risk, which is likely attributable, at least in part, to failures in the MMR system. Nonetheless, it must be considered that malfunctions in a single DNA repair pathway can be offset by alternative pathways, implying that simultaneous flaws in these compensatory pathways may lead to synthetic lethality. Consequently, identifying defects that manifest in mutually exclusive patterns can be utilized for the treatment of tumors with deficiencies in DNA repair mechanisms. This approach leverages the concept that targeting multiple compensating pathways concurrently could be an effective strategy for treating DNA repair-defective tumors.

Gastrointestinal malignancies, especially colorectal cancer, exhibit frequent silencing of HRR and MMR pathways, contributing to a high mutational burden. MSI tumors, constituting about 15% of colorectal and 10% of gastric tumors, result from defects in the MMR system, exhibit a slightly better prognosis, and respond to immune checkpoint blockade therapy. MSS tumors, constituting the majority, are characterized by chromosomal instability, show resistance to immune checkpoint blockade therapy, and present a challenge in terms of treatment options. However, the prevalence and prognostic roles of HRD in relation to MMRd in cancer require further exploration. Recent findings have unveiled a mutual exclusivity between MMRd and HRD mutational signatures in colorectal and stomach cancers[13], aligning with previous reports in gynecological malignancies[12] while offering valuable biological insights into the intricate relationship between the MSI/MSS status of tumors and the presence of HRD. It was observed that MSS tumors exhibited a higher degree of heterogeneity in their mutational signatures compared to MSI tumors: namely, MSI tumors usually showcase Single Base Substitution (SBS) signatures that distinctly represent a robust indication of MMRd, dominating the mutational signature profiles and suggesting potential selective advantages of these signatures which imply a driver role in shaping the mutational landscape of tumors.

Breast cancer (BC)

In BC, MMR genetic alterations are rare, occurring in 3% of cases, with a substantial intratumor heterogeneity. Notably, some studies have shown that breast cancers developed in women with LS are more likely to exhibit MMR protein loss and/or MSI, compared with sporadic tumors[72]. However, whether BC developing in the context of LS are causally related to MMRd, remains controversial. The existing literature on BC highlights how the tissue spectrum of LS patients may vary depending on the specific gene affected. Carriers of mutations in *MSH6* and *PMS2* have been reported to have an elevated risk of BC, in contrast to *MLH1* and *MSH2* carriers[73]. Despite the prognostic value of MMR is still controversial, several studies confirmed that MMRd is significantly associated with the worst prognosis and, especially in hormone receptor-positive patients, with an endocrine-resistant phenotype potentially susceptible to cyclin-dependent kinase 4/6 inhibition[74,75]. Regarding molecular subtypes, the distribution of MMRd BC is more prevalent in HER2-enriched and triple-negative breast cancer (TNBC) subtypes compared to luminal BCs[76]. In TNBC, the evaluation of both mismatch repair defect and TILs could be useful for selecting PD-L1-negative patients likely responding to immunotherapy[77]. For individuals with hormone receptor-positive BC undergoing tamoxifen treatment, the presence of MMRd is associated with poorer OS and DSS outcomes (HR 2.29, 95% CI 1.02–5.17, $P = 0.040$ and HR 2.71, 95% CI 1.00–7.35, $P = 0.042$, respectively). This observation implies that the MMR status might have a potential role in identifying hormone receptor-positive patients who might derive greater benefit from treatments other than endocrine therapy[75].

Pancreatic cancer (PaC)

The interest in MMR deficiency in PaC derives from its predictive role as an agnostic biomarker for immunotherapy. The prevalence of MSI in PaC is relatively low, accounting for around 2% of all cases[78]. The intraductal papillary mucinous neoplasm (IPMN) of the pancreas differs from its invasive counterpart for an incidence of 6.9% of MSI/MMRd[79]. Instead, pancreatic ductal adenocarcinoma (PDAC) and MMRd are associated in rare cases (1–2%)[78,80–82]. Luchini et al.[82] represented the most extended evaluation of this issue. MMR deficiency has been associated with a better prognosis, despite with not statistically significant data, and with mucinous/colloid histological phenotype. Of interest, *KRAS* is not the driver gene in this specific subtype population[81], whereas *JAK2-KMT2* gene mutations are frequently associated. Concerning the efficacy of immunotherapy, findings from the KEYNOTE-158 study indicated that MSI PaC exhibited a lower likelihood of response when compared to various other cancer types. Notably, the response rate to pembrolizumab in individuals with MSI pancreatic cancer stood at 18%, whereas response rates for other gastrointestinal cancers—such as gastric, bile duct, and small intestine cancers—were within the more robust range of 40%[83].

Prostate cancer (PrC)

In the context of prostate cancer, MSI is infrequently observed in the general population, and it does not represent a predominant pathway driving prostate carcinogenesis[84]. The majority of cases involve somatic mutations, with ~20% linked to LS, particularly in cases diagnosed before the age of 60[84]. Sporadic MSI prostate cancers are primarily associated with deactivating mutations in *MSH2* and *MSH6*, in contrast to colon and endometrial cancers, where MSI status arises through *MLH1* epigenetic silencing[85]. The activation of androgen receptor (AR) is implicated in the development of sporadic MSI prostate cancers, contributing to DNA DSBs[86]. MMRd is observed in 5% of metastatic PaC patients and is even less common in locally confined disease, with nearly half of MSI tumors presenting with metastatic disease. Comparative analyses of primary hormone-naïve tumors and their corresponding castration-resistant metastatic counterparts have revealed focal MMRd in the primary disease, suggesting that MMRd in the advanced setting may develop through clonal selection. Mutations in the *MSH2* gene have been identified as the most prevalent, although *MSH6* loss is more frequent in certain studies. Histologically, MSI has been detected in both adenocarcinomas and pure small-cell carcinomas, typically associated with aggressive disease, high-grade pathology, and metastasis. *MSH2* loss is correlated with dense CD8+ lymphocytic infiltration and a higher mutational load[86]. MSI is also associated with intraductal carcinoma and simultaneous *TP53* alterations. Clinically, MSI tumors exhibit a favorable response to androgen deprivation therapy and moderate sensitivity to docetaxel compared to MMR-proficient tumors (pMMR). Notably, patients with MMRd or MSI prostate cancer demonstrate significant responses to the PD-1 inhibitor pembrolizumab. In their study of 127 patients with castration-resistant prostate cancer, Rodrigues et al.[87] showed that MMRd was linked to a reduced median overall survival (mOS, 7.0 years for MMRp vs. 3.8 years for MMRd; $P = 0.003$), suggesting the negative prognostic significance of the MSI status in this setting. Activating mutations in the *MAPK* pathway, *PI3K* pathway, and *WNT/b-catenin* pathway were common[88]. About 50% of primary PrC exhibit *ETS* rearrangements, with *TMPRSS2::ERG* fusion being the most prevalent[89]. In MSI/MMRd-prostate cancer, however, TMPRSS2::ERG rearrangements seemed to be less represented.

Ovarian cancer (OC)

Previously reported frequencies of MSI in OC, based on individual locus assays rather than genome-wide searches, were ~10–12%[90]. However, when using a classifier that replicates the Bethesda MSI label through genomics, only 3.2% of ovarian tumors were classified as MSI[91]. This suggests that the commonly employed Bethesda panel-based readout might underestimate the prevalence of MSI in certain cancer types, such as OC, as well as head-and-neck and cervical cancer. A recent systematic review and meta-analysis reported that MMRd by immunohistochemistry and MSI analysis were detected in 6.7% and 10.4% cases, respectively, with a prevalence in endometrioid histotype and a 47% of cases with germline MMRd[92]. Diagnosis of OC with MMRd occurs at a median age of 52.3 years (interval 33.6–62.2), with an early stage mostly stage I (50%)[93]. The MMR deficient status is homogeneous in the entire tumor mass, suggesting an early inactivation in tumorigenesis in OC[94]. The association between MMR status and clinical features in Asian patients has been reported by Ye et al.[95] showing a higher rate of MMRd in women affected by ovarian carcinoma, with ≤50 years and a slightly higher median progression-free survival (PFS) than in their intact counterparts (30 vs 27 months), without a statistical significance ($P = 0.471$). OC in LS carriers exhibit a distinct profile compared to OC in individuals carrying *BRCA1/2* PV/LPVs[96]. The observed risk of mortality from gynecologic cancer diagnosed before the age of 40 in carriers of MMR PVs was found to be 0%. Consequently, it has been concluded that the practice of prophylactic hysterectomy and/or oophorectomy before the age of 40 solely for cancer prevention reasons is unwarranted and ethically questionable[97]. Similarly, the observed risk of mortality from OC in carriers of pathogenic *MSH6* or pathogenic *PMS2* variants diagnosed before the age of 50 was also found to be 0%. For these carriers, the recommendation is that prophylactic oophorectomy before the age of 50 solely for cancer prevention reasons is considered unwarranted and ethically questionable.

HRD signature in non-common HRD-spectrum tumors

Pan-cancer analyses from The Cancer Genome Atlas (TCGA) dataset have revealed that HRD impacts more than 5% of tumors, beyond the traditionally recognized HRD-spectrum cancers[98]. Notably, mutations in canonical HRR genes, typically associated with breast, ovarian, pancreatic and prostate cancers, have also been identified in a diverse range of cancers including colorectal (7–12%), esophagogastric (7.7%), hepatobiliary (6.6%), melanoma (18–57%), non-melanoma skin (10.5%), lung (6.3%), kidney (4.4%), endometrial (12.1%), and bladder cancer (10.0%)[99–101].

In a recent study on CRC, the presence of tumor deleterious variant in 33 genes involved in the HRR pathway was evaluated[102]. HRD tumors, defined as samples with 1 or more PV/LPVs, accounted for ~10% of MSS/pMMR CRCs, were more frequently TMB-high and PD-L1 positive, with important therapeutic implications In the same research, the analysis of the association between HRR alterations and genomic LOH in an independent cohort of CRC samples, showed that only HRD tumors in the MSS/pMMR group were LOH-high[102]. Both esophagogastric and colorectal cancers with HRD signatures are significantly associated with improved responses to platinum-based chemotherapy, attributed to the susceptibility of HRD tumors to DNA-damaging agents. This insight has spurred the development of clinical trials investigating the synergistic effects of combining platinum-based chemotherapy with PARPi[103]. Furthermore, the presence of HRD has been associated with elevated immune infiltration and TMB, critical determinants of efficacy for ICIs[102]. These discoveries establish HRD as a pivotal biomarker for guiding a range of therapeutic strategies—including chemotherapy, immunotherapy, and targeted therapy in gastrointestinal cancers, potentially enhancing survival for patients with specific genomic features linked to HRD[104,105].

In lung cancer, particularly non-small-cell lung cancer (NSCLC), HRD is emerging as an important factor in predicting treatment responses. Studies suggest that tumors with HRD may respond more favorably to immuno-neoadjuvant therapy, highlighting HRD status as a key marker for assessing the effectiveness of combined ICIs and chemotherapy[106,107]. Moreover, HRD serves not only as a predictive biomarker but also as a prognostic biomarker in lung adenocarcinoma, clear cell renal cell carcinoma (ccRCC) and endometrial cancer, where a high HRD score is associated with poorer outcomes[108–110]. Specifically, in ccRCC, genomic and transcriptomic analyses have shown that HRD-positive patients exhibit upregulated DNA damage response and immune-related signaling pathways[110].

Overall, the expanding recognition of HRD in a wider array of tumor types highlights its potential as an agnostic biomarker for cancer management, guiding more personalized treatment approaches and influencing the development of new therapeutic strategies tailored to exploit this genomic instability signature.

HRR and MMR in DNA repair: dichotomy or synergism?

MMR and HRR pathways could be, at the molecular level, closely linked[111]. Interestingly, even if not still fully clarified, several preclinical studies highlighted the crosstalk between MMR and HRR actors, with some proposed functions of MMR proteins during specific steps of HRR. In 2023, the workgroup headed by K. Myung highlighted the possible involvement of MMR protein complexes such as MSH2 and its partners (MSH3 or MSH6) and common proteins such as exonuclease 1 (Exo1) and SMARCAD1 (SWI/SNF-related Matrix-Associated Actin-Dependent Regulator of Chromatin Subfamily A containing DEAD/H Box1) in the regulation of HRR. In particular, it seems that the complex MRE11-RAD50-NBS1, after a proper recognition of DSB sites, generally followed by an ATM-dependent DNA damage signal, degrades ssDNA by generating a short single-stranded gap which in turn can be recognized by the heterodimer MSH2-MSH3 facilitating the action of Exo1 which can initiate DNA end resection by generating a longer ssDNA through its 5'–3' exonuclease activity (Fig. 2).

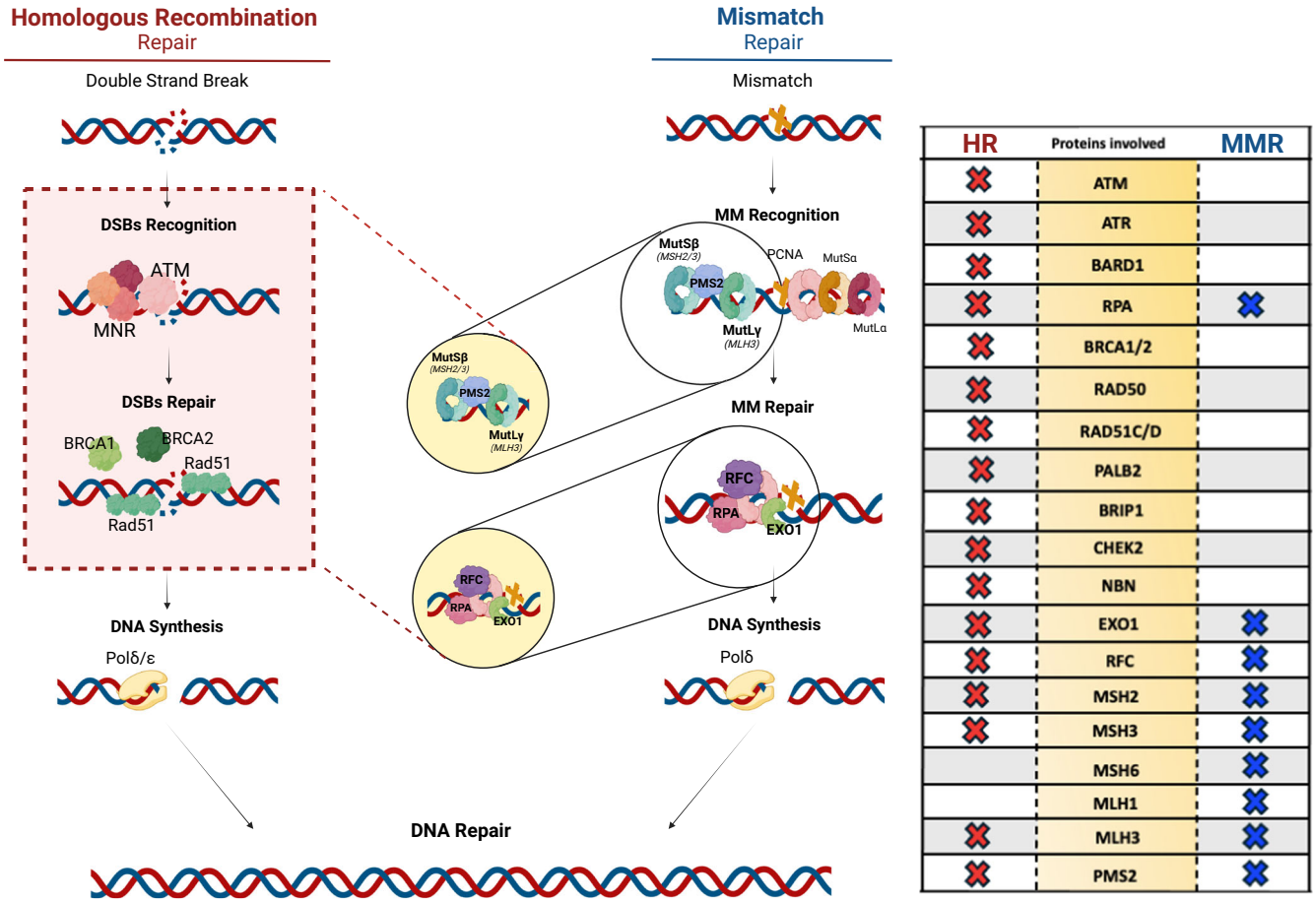

Fig. 2 | The link between MMR and HRR. Emerging studies highlight potential interactions of mechanisms that underly different forms of DNA repair, such as MMR and HRR. Shared core proteins, including RPA, EXO1, RFC, MSH2/3, MLH3, and PMS2, are identified as key players in both pathways, broadening the concept of DNA repair mechanism exclusivity in specific tumor types (Created with BioRender.com).

The recruitment of MSH2-MSH3 to DSBs could be facilitated as a result of a fine chromatin unwinding process in the nearby DSBs operated by remodeling complexes such as SMARCAD1. Indeed, SMARCAD1 has shown highly conserved interaction domains with MSH2. In addition, another finding reported the blockade of DNA polymerase θ-mediated end joining (TMEJ) by the heterodimer MSH2-MSH3 preventing consequently the misincorporation of errors during HR[112].

Recent studies further elucidate the intricate synergistic cooperation between MMR and HR pathways. Interestingly, as demonstrated by the research group headed by Yang-Xin Fu and Guo-Min Li, MutLα subunit MLH1 deficient mice show a higher chromosomal instability. In particular, MLH1 regulates Exo1 nuclease activity during DNA repair, and loss of MLH1 causes unrestrained DNA excision by Exo1, leading to increased single-strand DNA formation, DNA breaks, and, ultimately, chromosomal instability. This mechanism activates the cGAS-STING pathway, with important clinical implications for cancer immunotherapy[113].

Moreover, HRR contributes to fork maintenance and the repair of spontaneous and induced DSBs[111]. The proteins involved in both systems partly overlap, up to now the molecular mechanisms underlying the activity of this system are only partially known but their cooperation is already demonstrated[111]. Bacterial but also yeast and mammalian cell models have highlighted the involvement of MMR proteins, in particular, the heterodimer MSH2-MSH6, in the interruption of recombination products generated from the genetic exchange between not perfectly homologous DNA strands in a process termed homeologous recombination[111].

Furthermore, a recent finding highlighted the pivotal role of MutSβ, which corresponds to the heterodimer MSH2 and MSH3, in modulating HRR-mediated repair by resolving Holliday junctions (HJs). Specifically, the interaction of MutSβ with the SMX complex, comprising SLX4-SLX1, MUS81-EME1, and XPF-ERCC1, introduces a novel perspective on HRR regulation. MutSβ directly interacts with SLX4, a central component of the SMX complex, suggesting a targeted influence on the resolution of critical intermediates in HRR. Such findings further highlight the complex interplay between MMR and HRR pathways, extending their role beyond mere suppression or correction of mismatches through close collaboration in resolving recombination intermediates, thereby ensuring accurate and efficient DSB repair[114].

Despite these preliminary evidence, elucidation of how this partial crosslink and crosstalk is regulated, and its full biological and clinical implications, require further research.

Clinical insights on HRR-MMR intersection: therapeutic consequences

The interactions of mechanisms that underly different forms of DNA repair could make the potential use of PARPi and ICIs significantly broader than initially recognized.

The relationships between HRR and MMR were recently investigated in gynecological cancers[12]. As well known, MMRd and HRD are distinctive signatures of uterine endometrial carcinoma and epithelial ovarian carcinoma, respectively. Farmanbar et al.[12] studied the mutational signature profiles in gynecological cancers. A pattern of mutual exclusivity of DNA repair pathways was observed: a subset of uterine endometrial tumors showed HRD, a subset of epithelial ovarian tumors showed MMRd signature, while, in a cohort of cervical tumors, APOBEC was the most prevalent signature, co-occurred with POLE and was mutually exclusive with the MMRd signatures[12]. The authors concluded by highlighting a potential

cancer type-independent ternary relation between HRD, MMRd, and APOBEC, where MMRd mutational signature is mutually exclusive with HRD, and APOBEC co-occurring with HRD is mutually exclusive with MMRd[12].

The following research data were consistent with the reports on gynecological cancers. Mutual exclusivity of MMRd and HRD mutational signatures in colorectal and gastric tumors was shown. However, in the context of MSS tumors, a distinct subset of HRD tumors, characterized by poor outcome, was identified. Because HRD is a predictive biomarker of PARPi response, this finding could have important implications for therapy management of MSS tumors[13]. Furthermore, preclinical studies have suggested that MSI-mediated loss of DSB repair genes could confer sensitivity to PARPi also in MMRd cells[115,116]. Future studies in clinical setting confirming these data could open the way to novel therapeutic opportunities, beyond the immunotherapy, for the treatment of MMRd-related cancers. A following study of Sokol et al.[117] explored the genomic overlap of MSI with the LOH, as a genomic measure of HRD, and the *BRCA1/2* variant zygosity across multiple tumor types. The results highlighted that MSI and HRD status were generally mutually exclusive phenomena across breast, ovarian, and pancreatic tumors, with rare co-occurrence of *BRCA* mutations in the context of MSI. Notably, in prostate cancers, 12.8% of *BRCA1* and 3.4% of *BRCA2* mutations co-occurred with MSI. However, in these tumors, the *BRCA1/2* mutations were generally monoallelic and were not associated with high LOH scores, ultimately not leading to an HRD phenotype[117]. Despite the authors suggesting less benefit from PARPi than ICIs, the small number of patients with combined *BRCA* mutations and MSI status does not allow any clinical recommendation in this subgroup of patients. A recent phase III clinical trial explored whether HRD-positive ovarian cancers patients benefitted from atezolizumab (IMagyn050 Trial, NCT03038100). The results showed that most ovarian cancers had low TMB despite HRD, and the presence of genomic instability did not improve sensitivity to ICI atezolizumab[118]. Thus, future efforts in clinical context are needed to further elucidate the clinical impact of genomic integrity maintaining mechanisms intersection, and to introduce novel concepts and hypotheses for novel therapeutic opportunities.

In conclusion, the number of innovative cancer therapies on the basis of the genetic background and genomic profile has dramatically increased in the last few years. Defective DNA repair processes are among the main targets for cancer therapeutics. HRR and MMR seem to have biological points of intersection. The clinical effects of this interaction remains unclear. Although MMRd and HRD were often identified as mutually exclusive genetic phenomena, recent evidences suggest that sensitivity to PARPi could be not associated with HRR defects alone[115,116]. HRD could occur in the context of MSI status, MMRd or MSS tumors, resulting in potential PARPi benefit. The attention is now turning to in depth studying mutational imprints of DNA damage also in non-canonical HRD or MMRd/MSI tumors, considerably increasing the opportunities for targeting DNA repair-defective cancers.

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Competing interests

The authors declare no competing interests.

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